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THE RELATION OF THE INDUCED MUTATION RATE TO DIFFERENT PHYSIOLOGICAL STATES IN DROSOPHILA MELANOGASTER: III. IRRADIATION DURING RAPID AS COMPARED WITH SLOW GERM-CELL FORMATION[1]

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OBSERVATIONS reported from time to time by numerous investigators have brought evidence that rapidly growing tissues are particularly susceptible to the effects of irradiation. The first law of radiation therapy, formulated by Bergonié and Tribondeau (1)[2] in 1906, stressed the sensitivity of cells in an active state of division. This generalization was the result of extensive studies upon adult and embryonic cells. More recent investigations have confirmed the susceptibility of cells undergoing division, although there is some divergence of opinion as to which is the critical mitotic phase. Regaud, in 1923 (8), recorded two maxima during the process, the prophase and the anaphase. Strangeways and Hopwood in 1926 (9), studying the effects of x-rays upon mitotic divisions in vitro, considered the brief period of organization just before division the high point of sensitivity. Vintemberger (10) concludes that susceptibility gradually increases as mitosis proceeds and then falls sharply at the telophase.

[1] The expenses incurred in connection with this investigation were supported in part by a grant from the committee on the effects of radiation upon living organisms of the National Research Council.

[2] Items of the literature cited in the first two papers of the series are referred to by number.

Hertwig ('11), investigating nuclear changes in irradiated ova of Ascaris, recorded maximum sensitivity when divisions were taking place in rapid succession. These findings received independent corroboration from experiments conducted by Mottram ('13) with a view to determining whether the various stages in the life-cycle of the developing ovum showed changes in vulnerability to the same amount of radiation. He exposed ova in different stages to the beta and gamma-rays of radium and found them to be on the average about eight times more sensitive when in active division than they were if in resting condition. At ten minutes' exposure 99 per cent. of dividing ova were killed, while 150 minutes were necessary to kill 100 per cent. of resting cells. Mottram considered the metaphase a particularly sensitive stage. Regaud, in 1922, in cytological studies on the testis of the cat, observed periodic increase in sensitivity corresponding to the rhythm of mitotic activity.

Some investigations have indicated not only high sensitivity of dividing cells, but at the same time changes in the rhythm of mitosis, especially a definite stimulation of division by light doses, such that radiation effects within a certain range of severity might be inter-reactive. Marked acceleration of the mitotic rate was observed by Lazarus-Barlow and Beckton ('13) in the eggs of Ascaris, but the margin between exposures under which division proceeded at an increased rate and those under which it was progressively retarded was found to be very narrow. In tissue culture work these phenomena have been studied to particular advantage. Regaud, Lacassagne and Jovin (8), working with x-rays found that in chick embryos a temporary check in cell division was followed by a considerable increase in the number of dividing cells.

Interesting studies have been made of radiation effects upon vegetable life: upon seeds and seedlings in various stages, upon leaves and budding twigs, and upon the fully developed plant. The same phenomenon of acceleration under slight exposures has been noted. Falta

and Schwarz ('11) exposed oats to weak radium emanation and found their growth accelerated. By suitable irradiation Molisch ('12) was able to hinder or favor the growth of buds according as they were in resting or growing condition. Buds of *Syringa vulgaris* given slight exposures in the resting stage opened normally and sooner than controls, but those already in the growing stage at the time of irradiation suffered harm from the same exposure. Molisch considered the acceleration effects of irradiation the same as those of forced growth by temperature, *i.e.*, the hot-house method. Lacassagne and Holweck ('30), however, studying the radiosensitivity of yeast (*Saccharomyces ellipsoideus*) found no stimulating action to budding resulting from exposure to x-rays or the alpha-rays of polonium, and no increased sensibility associated with reproductive activity.

Stadler ('28[a], '28[b]) has conducted experiments with barley in which he finds the induced mutation rate in the growing apical buds of sprouting seedlings to be approximately eight times as high as that of resting seeds, an interesting confirmation numerically of results obtained by Mottram ('13) with rapidly dividing animal tissues. These observations are paralleled in studies of malignant tissues. As a result of detailed histological examination of irradiated tumor tissues, Contamin ('09, '10) came to the conclusion that the action of x-rays is greater the younger and more actively growing the tumor is.

From such findings the general consideration seems to follow that sensitivity is increased wherever cellular changes are in progress, wherever the physiological characters are not fixed and the cell constituents are in a state of flux.

Experimental Results

The present authors in connection with experiments dealing with the physiology of mutation-production (papers in press at the time of this writing) have made a study of the effect of exactly the same radium radiation upon impregnated females of Drosophila in which oögo-

nial division and multiplication is known to go on at a rapid rate and virgin females in which oögonial activity is at relatively low ebb.

First generation bar-eyed females of the cross, C1B (sc,v,f,bb) by wild, were selected for treatment. It will be recalled that such females, having one x-chromosome carrying the C1B factors and one x-chromosome the wild-type, are heterozygous for the lethal character. When mated to the wild-type they give one half the usual number of sons in the F_2 generation or a 100:50 sex ratio. The sc,v,f,bb-chromosome from the P_1 female goes to the first generation sons which in these experiments are discarded. A second lethal mutation induced in the chromosome carrying the wild-type characteristics is transmitted to the other half of the sons, and they also fail to appear. As has been described in the previous papers preliminary dosage experiments demonstrated the occurrence of such an induced lethal in this x-chromosome in proportion to the dosage applied.

By the work of Hanson and Ferris ('29) on fecundity in Drosophila it was shown that the egg-laying capacity of mated females is much greater than that of virgins. The peak of fecundity was found to be reached early by the mated female and later by the virgin. The greatest daily average per female is reached on the fourth day by mated females and the seventh by virgins. As indicated by experiments with sterilized males, the presence of the male was found to exert a "physical or psychical stimulus" to greater egg production. It seems reasonable to suppose that oögonial divisions must be going on at the most rapid rate just previous to the peak of fecundity.

With this in mind the females irradiated in the present experiment were divided into two groups. Immediately after hatching one half were mated to wild-type males for a period of three days, while the others which remained virgin were isolated for exactly the same time. At the end of this three-day period both groups were exposed to a radiation of 300 milligram-hours, gamma radiation only, at a distance of 10 cm. Alpha and beta-rays were

excluded by filtration through 0.5 mm. gold capsules enclosing the radium-element. Supplementary filtration was of two kinds: sheet lead, of a thickness of 1 mm, and a layer of Columbia paste, 1 cm in thickness. While the set-up here differed slightly from that of previous experiments the total filtration used allows the passage of exactly the same percentage of the gamma radiation as before: gold of 0.5 mm allows 79 per cent.; lead of 1 mm allows 78 per cent. After treatment all females were mated to newly hatched wild-type males.

A comparison of the results obtained in the two groups of irradiated females is given in Table I. Table II shows

TABLE I

SHOWING THE PERCENTAGES OF STERILITY AND LETHAL MUTATION FOLLOWING IRRADIATION OF MATED FEMALES AS COMPARED WITH VIRGINS

Impregnated Females	Virgin Females	Difference	Significance
	Per cent. of Sterility		
14.2800 ± 0.4606	8.2000 ± 0.3701	+ 6.0800 ± 0.5808	10.47 × p.e.
	Per cent. of Lethal Mutation		
14.4395 ± 0.5224	8.5403 ± 0.3935	+ 5.8992 ± 0.6540	9.02 × p.e.

the number of cultures upon which the percentages are based. Figure I is a graphic representation. Fertility tests were made in the same manner as in previous experiments, so that the sterility values given here represent, as before, complete sterility. The increased sterility and the high rate of mutation obtained in females irradiated after impregnation are striking, the differences being sufficiently great to bring them well within the range of significance.

While sorting out cultures to test those in which nonfertility was suspected, it was noticed that a number of the mated-females series showed pupae as normally, but that no flies hatched. In these a period of about five days' delay (average—5.0367 ± 1.4320) occurred, but

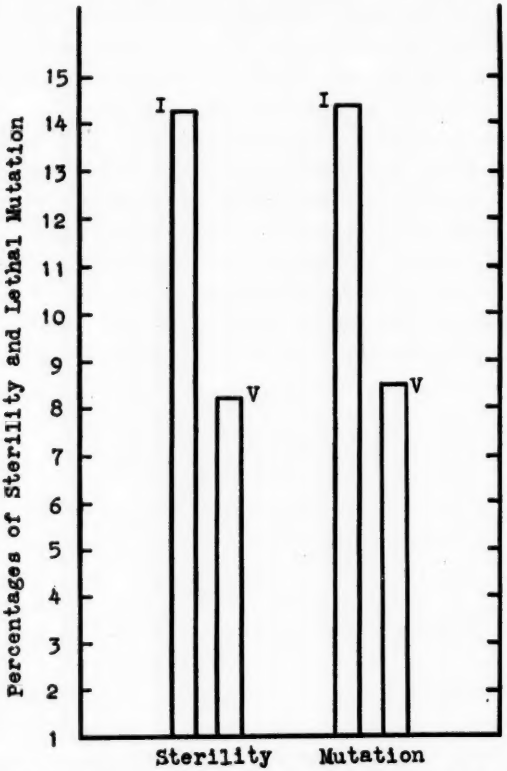

FIG. I. Showing percentages of sterility and lethal mutation. I = impregnated females; V = virgin females.

TABLE II

GIVING THE NUMBER OF CULTURES UPON WHICH PERCENTAGES OF STERILITY AND LETHAL MUTATION IN TABLE I ARE BASED

Total No. Cultures	Number Sterile	No. Fertile Cultures	No. Lethal Mutations
	Impregnated Females		
2500	457	2043	295
	Virgin Females		
2500	205	2295	196

afterward the cultures hatched, some giving both males and females and some bar-eyed females only. In a small percentage, 2.7322 ± 1.7603, males and only round-eyed females hatched for a period of about four days (average -3.9670 ± 1.5723), and after four days males, bar-eyed females and round-eyed females.

Altogether in 2,500 matings in the impregnated-females series 183 such cultures occurred, or a percentage of 8.9574 ± 0.4194. This value corresponds very closely to the mutation value for treated males in other experiments, 8.9887 ± 1.4451. Consequently it seems logical to attribute these results to the induction of a lethal mutation in sperm carried by the impregnated females. If a lethal mutation were induced in these sperm, the females having already the lethal character carried by the C1B-chromosome would inherit it and fail to appear. If at the same time an induced mutation occurred in the x-chromosome carrying the wild-type factors, no flies at all would result from eggs fertilized by sperm irradiated in the seminal vesicles of impregnated females. Figure II is a scheme of mating showing the possible chromosomes in which lethals might have occurred and giving the consequent results in the next generation: A explains those cases in which lethal mutations were induced simultaneously in the wild-type chromosome of the female and in the sperm carried by that female. For a period of about five days no flies hatched, but after that time the cultures hatched, some giving both males and females and some bar-eyed females only. B explains those cases in which a lethal mutation occurred in the sperm carried by the female, but none in the wild-type chromosome of that female. For a period of about four days males and only round-eyed females hatched, and after four days, males, bar-eyed females and round-eyed females.

After recovery 26 of the above 183 gave only females, a mutation rate of 14.2765 ± 1.7439. In other experiments in which the C1B stock has been used it was noticed that some pupa cases appeared still opaque in

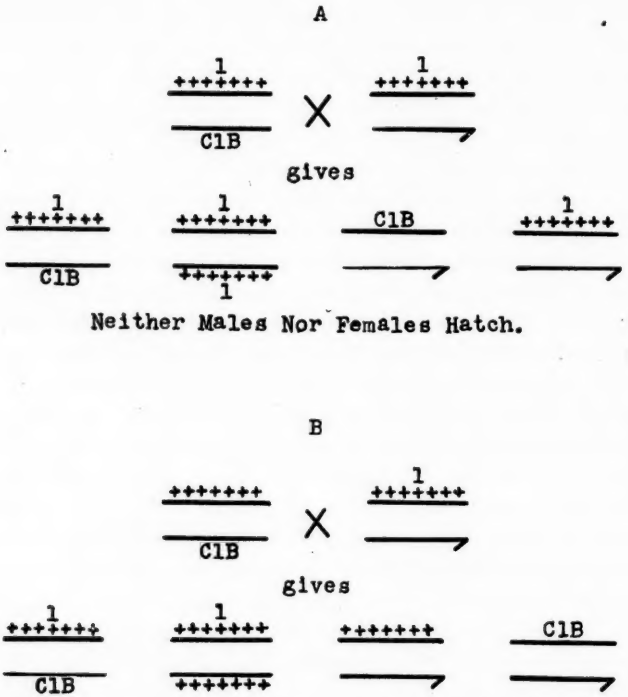

FIG. II. Scheme of mating showing the possible chromosomes in which lethals might have occurred.

contrast to the transparent empty cases, when all flies had hatched, but no counts were made as in the present experiments. The lethal seems, therefore, to be one which is effective in the pupal stage.

DISCUSSION

On first examination of these observations it appears that there is perhaps some overlapping of irradiated and non-irradiated sperm and that a part of the high sterility and mutation values in females irradiated after impreg-

nation might be due to lethals induced in the irradiated sperm. However, the delay in hatching and the sharp demarcation later between those cultures which gave no flies at all and those which gave females only, bar-eyed, round-eyed, or both, would seem to indicate that the mixture of the two kinds of sperm was not great. The wild-type males to which irradiated impregnated females were mated were newly hatched and would require some hours to reach full maturity. Making allowance for possible overlapping, the differences in the results are still sufficiently great for significance. Since these differences are apparent when mated females irradiated just previous to the height of fecundity are compared with irradiated virgins in which productivity is at relatively low ebb, they may be explained perhaps by differences in radio-sensitivity due to rapid cell multiplication in one case and not in the other.

Summary

Previous work (Hanson and Ferris '29) by means of daily egg counts had indicated that mated females laid an enormously greater number of eggs than virgins. The experiment reported in this paper deals with the effects of exactly the same radium radiation upon impregnated females in which oögonial division is going on at a rapid rate and virgin females of the same age in which oögonial activity is at relatively low ebb. It is generally accepted that somatic cells (observations in tissue culture work, on malignant tissues, etc.) undergoing rapid cell division are highly susceptible to radiation effects. The results of this experiment seem to indicate that the same is true for germ cells. In the series of irradiated virgins the sterility and mutation rates are significantly lower than in the mated series where the rate of germ-cell formation is much higher.

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A STUDY OF DOMINANT MOSAIC EYE-COLOR MUTANTS IN *DROSOPHILA MELANOGASTER*

I. PHENOTYPES AND LOCI INVOLVED

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ALLELOMORPHS OF BROWN

THE writer has been working for the past two years upon the inheritance of variegated eyes in *Drosophila melanogaster*. Most of the characters studied form a series of dominant allelomorphs, lethal when homozygous, of the gene brown (bw), which is located between plexus (px—99.8) and speck (sp—106.3) in the extreme right end of chromosome II, at locus 104.3. Several of these, including "Plum," "Discolored," and "Tarnished," have been previously figured and described by Muller ('30b). Three others, similar in appearance, and known as "Rosy," "143a," and "A34," were likewise obtained after x-ray treatment, the two former by Patterson and the latter by Moore. These six, after the order of their discovery, have been given the symbols bw^{V1} (Plum), bw^{V2} (Discolored), bw^{V3} (Tarnished), bw^{V4} (Rosy), bw^{V5} (143a), and bw^{V6} (A34); but since in the present paper it can be understood that they are at the locus of brown, these are abbreviated to V1, V2, etc.

In appearance these are all similar to Plum as described by Muller ('30b). The color of the eye changes from a yellowish-brown on emergence to a bluish-purple. "The pigmentation is not uniform throughout the eye, but shows darker and lighter patches of variable size, seen best in the older flies, but also noticeable in the young flies in the deeper layers of the eye when the upper layers are shaded." It is impossible to distinguish individuals of these separate allelomorphs with certainty; yet there are phenotypic differences between them.

Plum is more of a homogeneous color than Tarnished, in which the specks of darker pigmentation are often discrete and plain. Rosy is more similar to Plum, and V5 and V6 are nearer the appearance of Tarnished. Yet overlapping individuals are found in each group which make accurate differentiation impossible. When combined with homozygous scarlet, (III—44.0), the pigmentation is light orange in color, with specks of darker orange which may be so numerous in some individuals as to merge in irregular areas, and apparently form the ground-color of the eye. This form, in all cases, is quite similar to the appearance of Discolored when combined with homozygous vermilion (v—X—33.0), as figured by Muller ('30b); but the specks may be much more numerous in some individuals than in others, and indeed are less numerous in Discolored than in most of the other allelomorphs. Nor have two distinct forms, such as Muller described for Discolored, been obtained with other members of the series. The pigmentation varies considerably in amount, from individual to individual, but the general appearance remains quite similar.

Each of the six allelomorphs is very nearly always lethal when homozygous (over 90 per cent.). The lethality depends largely on culture conditions, some cultures giving numbers of homozygotes, while others fail to produce any. These homozygotes have an eye-color which is brown, and apparently quite homogeneous. They are fertile, and have occurred in all the stocks. Their wings often fail to unfold, and when they do present a peculiar crepe-like texture. When the homozygous variegated allelomorphs of brown are combined with homozygous scarlet, the eye is white, but occasionally shows a vestige of the light orange pigment in isolated ommatidia. Such white-eyed females, when mated to homozygous scarlet males, produce only offspring having the eye-color characteristic of the combination of dominant variegated brown with homozygous scarlet, a fact showing that they are indeed homozygotes.

Johannsen ('24) has described the presence of pigment granules of two colors in the eye of Drosophila. These are respectively "a wine-purplish red and an ochre-yellow"; or as Casteel ('29) terms them, "maroon" and "yellow." Removal of the maroon granules leaves the yellow ones, which in large numbers presumably produce a scarlet or vermilion color, as in the case of red blood corpuscles. The effect of the genes scarlet and vermilion, and others like them, is therefore principally to remove the maroon pigment; while brown and its allelomorphs are among those genes which principally affect the presence and quantity of the yellow pigment granules. Two doses of one of these dominant allelomorphs of brown are usually sufficient to remove the yellow pigment entirely, but one dose removes only a portion of it.

It has been found in our own laboratory (Moore, unpublished data), and by Wright ('32) that recessive bw, when homozygous, is sufficient to remove all the yellow pigment, producing, in combination with homozygous st or v a pure white eye. The dominant allelomorph, then, in heterozygous condition, is not as powerful in action as is the homozygous recessive, although the heterozygous recessive gene is apparently below the threshold of reactivity and produces no effect on the eye pigmentation whatever. The dominant allelomorph in homozygous condition is approximately equal in effect to the homozygous recessive, but in some cases, as was mentioned above, single *ommatidia* show traces of the yellow pigment. Whether this means that the homozygous dominant is actually weaker in action in some areas than the homozygous recessive; or whether it means that reverse changes, either mutational or chromosomal, have occurred in isolated *ommatidia,* from bw to normal, is yet uncertain.

It has been found by Bridges (*ex lit.*) that Plum, when present in single dose in a hyperploid fly carrying as excess the right end of chromosome II (supplied by means of crosses with his Pale translocation), failed to mani-

fest its dominant color. It was therefore evident that these hyperploids carried two doses of non-Plum and that the locus of Plum must be in the region carried as excess, *i.e.*, the right end of II. Following this, we found that the same phenomenon was true of the other five characters of the series. These are therefore in the same region. (It may be mentioned, however, that although the color of the eye appeared not definitely abnormal in the hyperploids, traces of the variegation were still faintly visible.) A test was then devised by Muller to determine whether or not these mutants were allelomorphs of the recessive mutant brown, since brown is known to be in the region in question, and since it somewhat resembles them in color, although it is not variegated. This test involved the making up of a genotype containing one dose of the dominant variegated, one dose of the recessive brown, and the extra fragment containing a dose of the normal allelomorph (or allelomorphs). In each of the cases in which the earlier test had shown that the dominant became suppressed in the hyperploid, it was now able to manifest itself. It was therefore clear that all of these variegated genes were allelomorphs of brown and of each other. In addition, it was found that, in simple heterozygotes with brown, a solid eye-color was produced with no apparent variegation.

To further substantiate the allelomorphism, the stocks were intercrossed. Since each is maintained in balanced stock with the dominant gene for Curly wings (Cy—II) in the homologous second chromosome (along with inversions that prevent crossing-over), the appearance among the F_1 flies of individuals with normal wings would indicate that the combination of two variegateds in one fly was viable, and hence presumably not a "compound" of two allelomorphs. This did not prove to be the case; only flies with Curly wings appeared, excepting for a very small percentage of the abnormal looking "compounds," similar in appearance and equal in proportion

to the homozygotes appearing in the pure stocks of the six characters.

In view of these findings, a series of tests similar to the first mentioned (involving Pale translocation but not brown) was then carried out by Van Atta ('32), working with Oliver at Washington University, on a group of dominant eye-colors which she had been studying and had called "Dilutes." Since each of her six cases on analysis proved to be likewise located in the extreme right end of II, and since each one, lethal when homozygous, also produced only lethal combinations with the others, it is almost certain that they too are allelomorphs of brown.

The brown locus, therefore, is especially subject to lethal, dominant changes as a result of irradiation, and like the white locus, to changes having a somatically unstable spotted expression.

Grape

Grape is somewhat similar in appearance to the preceding group. It was found after x-ray treatment by Muller. It differs in presenting a practically homogeneous color, a rosy purple. With care, it may be distinguished from the members of the brown group by its appearance. Like them, it is almost entirely lethal when homozygous. When combined with homozygous scarlet, the eye-color is a light orange, but shows no distinct spots, the pigmentation varying gradually in intensity from one part of the eye to another. It is otherwise similar to the combinations of st with the dominant brown allelomorphs.

When tests were made with Bridges' Pale translocation, it was found that the hyperploids showed the eye-color characteristic of Grape. This means that the extra piece carried by the hyperploids, the extreme right end of II, fails to contain a counteracting normal allelomorph of Grape. When crossed to any of the six members of the preceding group, the hybrids are perfectly viable,

and have a homogeneous yellow-brown eye-color. Upon being crossed to pink (III—48.0), the heterozygotes showed a pinkish-orange eye-color, much lighter than that of ordinary heterozygous Grape. Considering this in connection with the tests to be described in the next paper of this series, in which the locus of Grape is shown by crossing-over tests to be either in the region covered by the extra piece of the Pale translocation, in II, or in III at approximately the locus of pink (these being the regions of the two chromosomal breaks), it is virtually certain that Grape is an allelomorph of pink.

MOIRÉ

Moiré 1 was found by Muller, and has been described and figured by him ('30b). The phenotypic appearance differs somewhat from that of the series of brown allelomorphs, in that in the present case the eye-color has a shimmery aspect. This is not well shown in the plate.

Moiré 2 was found by Moore. It is so similar in appearance to Moiré 1 as to be phenotypically indistinguishable from it. Like Mo^1, it is located in the left arm of chromosome III, but the exact location of either has been impossible.

The hybrids between Mo^1 and Mo^2, like the homozygous genotypes, are inviable. For when Mo^2/Cy flies are mated with Mo^1/Pr, only flies containing either Pr (Prickly—III—90.0) or Cy are found in the offspring. Cy can be used to balance Mo^2 because of a translocation between II and III (as will be discussed).

THE RELATIONSHIP OF DOMINANT MOSAICS TO BREAKS IN THE CHROMOSOMES

As Muller pointed out in the general discussion of his paper already cited, heritable somatic variegation produced in *Drosophila melanogaster* by irradiation has always been accompanied by breakage and reattachment of chromosomal parts; and dominant eye-colors have also usually involved breakage and reattachment. (Ex-

ceptions are dominant Garnet in *Dros. virilis* and Henna in *Dros. melanogaster,* the latter found by Van Atta ('32).) This general relationship has since been shown to be true both of variegated and of dominant eyes found after his paper was written. The breaks have been of the double type, so that upon reattachment we obtain either inversions or mutual translocations.[1] In the series of brown allelomorphs we have two cases, Plum and Discolored, which are inversions; and four which are mutual translocations. Grape is a mutual translocation. Moiré 1 is an inversion; while Moiré 2 is a mutual translocation.

In every case a breakage of the chromosomes has occurred at or very near the locus affected.

Summary

(1) Six dominant mosaic eye-color mutants of the brown locus (II—104.3) have been studied. All are over 90 per cent. lethal when homozygous. The homozygotes are characterized by nearly homogeneous eye-color, and by wing abnormalities. These allelomorphs, like brown itself, affect principally the quantity of the yellow pig-

[1] The writer wishes to advocate the use of the term "mutual" for such translocations as the above case, and A, B, and C of Dobzhansky and Sturtevant ('31), rather than the term "reciprocal." It would seem that "reciprocal" would apply more properly to a case in which two different translocations, when compared with one another, are seen to bear a reciprocal relation to one another, as when one translocation involves an attachment of II R to III and the other an attachment of III R to II. "Reciprocal" translocations might in some cases be mutual and in other cases simple translocations. To avoid confusion, we suggest that the term be used only in the latter sense. "Mutual" translocation implies the exchange of pieces between different chromosomes of the same individual. It is therefore the same as the term "segmental interchange" of Blakeslee and Belling, but unlike it, can not be understood as including crossing-over. "Mutual" possesses the advantage that it may be extended to apply to cases or systems in which more than two chromosomes are all similarly involved, as in the rings of Oenothera, Datura, or Rhoeo; while "reciprocal" would seem to be limited to two chromosomes only. As to the use of the two terms in the present situation, "mutual" seems to have the priority, having been first used by Muller ('30a).

ment of the eye, even removing it entirely when homozygous.

(2) Four of these six are associated with mutual translocations; two with inversions. In each case one of the breaks is at or very near the locus concerned.

(3) One dominant allelomorph (Grape) of the mutant gene for pink eye (III—48.0) has been studied. It, too, affects the amount of yellow pigment present in the eye. It is lethal when homozygous. It is associated with a mutual translocation, and one break is at or very near the locus concerned.

(4) Two dominant allelomorphic eye-colors (Moiré) located in the left eye of III are associated, one with an inversion, the other with a mutual translocation. These are also lethal when homozygous.

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RACIAL AND INDIVIDUAL VARIATION IN ANIMALS, ESPECIALLY FISHES

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I

JUST before the explosive evolution of genetics during the opening years of this century, the study of variation was growing with a promise of becoming a major subfield of zoology. This development of variation studies then became obscured, in part only apparently because of an overshadowing shift in biological attention, but largely in fact. The promise of a new field (an allurement in itself), the greater satisfaction in the newer experimental as contrasted with the older circumstantial evidence which had been gained in biometry, the greater definiteness of individual over population analysis—such factors attracted biologists from biometry into genetics. And with the natural pride and satisfaction in their epochal progress, the geneticists developed a feeling which often approached scorn toward the variation studies of the preceding generation.

The field of variation, following the principle of the phylogeny of animals, has continued to exist on, as an evolutionary strand during the flourishing rise of the derived field of genetics. Perhaps a new progressive evolutionary movement may sometime arise from the generalized relict.

In certain fields and for special purposes, the population analysis of biometry was not, or could not be displaced entirely by the individual experimentation of genetics. In human heredity, for example, genetic experiments have been inhibited by the slow breeding if not the unwillingness of the possible subjects, and recourse must still be had to the cruder, less efficient tool of observational statistics.

In the new systematics, likewise, genetics can not replace variation investigations. This is not because genetics does not broaden the general biological view of the systematist, nor because genetics may not offer him detailed data of actual value in his systematic interpretations—but because systematic groups are populations, and must be investigated by population analysis.

It has been in large part the increased use of statistical methods and the development of a statistical sense, in species and subspecies and local race discrimination, which has caused systematic zoology—I speak particularly for systematic ichthyology—to make distinct progress during the last half-century. To be sure many biologists (unacquainted with the systematic field, certain physiologists and geneticists for instance, still display their ignorance by insisting that no advance in systematics has taken place since the days of Linnaeus. I hold the view, perhaps in extreme contrast, that much the same order of difference exists between the fish systematics of so excellent a last-century ichthyologist, as for example Günther, and that of the coming if not of the present school, as contrasts the heredity of Galton with the genetics of Morgan. Not only is it becoming increasingly the habit of the systematist to search the exterior and even the interior of his animals for possible distinctive features, but he is also coming to consider distribution, habitat, habits and life history, and the characters of the young and even of the germ cells. But experience is clearly teaching the systematist that even such refined systematic analysis may only formulate his problems: to determine whether the observed differences are consistent he is often forced to obtain a much larger series of specimens than his predecessors would have considered accumulating, and to analyze statistically the variation in the characters of his many specimens, whether they be internal or external, of bone or of flesh, of anatomy or of color.

In the analysis of the local races of fishes, the variation

studies often become of such importance as to take a leading place in modern fisheries research, and of such general biological interest as to attract the evolutionist. Much indeed can often be accomplished by the critical application of the statistical method. The ichthyologist may by this means go far toward analyzing the local raciation within species of fishes; the range and movements of each stock; the characteristics of each; the individual variation within each racial unit, especially as these may be correlated with varying temperature, salinity, turbidity or other environmental factors operating at the critical period of development, or with such biological factors as early parasitization. By such means he may be able to obtain some estimate of the relative importance of racial and environmental factors in determining the characteristics of fish population, an important point now that it has been learned that all of these factors may alter, sometimes greatly, the structural and meristic characters of the individual.

An appreciation of the effect of environment in determining the characters of the fish (or other kind of animal) will no doubt often lead the student of fish races into a realization that again his problem is rather formulated than solved. A new and surer research tool should be used, namely *experiment.* A fine example of the use of experiment in race analysis is the work of Sumner (1924, 1926, 1932, etc.) on American mice of the genus *Peromyscus.*

The intrusion of the experimental method into systematic zoology I look on as a pioneering act of paramount significance in the certainly approaching revolution in systematics. An actual field of *experimental systematics* appears to be in process of organization.

Experiment in race analysis ought not to be regarded as a substitute for statistical analysis, any more than statistics can rightly (or effectively) be regarded as a substitute for non-mathematical systematics. Rather it is an additional tool. The analysis of natural populations

by the statistical study of samples will remain a virtual necessity.

Some fishes—tropical cyprinodonts form a notable example—are highly suited for experimental systematic research, since they breed so readily and rapidly in laboratory aquaria that they have been used even in genetic research. In others, as the salmonoids, easily propagated in hatcheries and reared in ponds, racial constancy and the effects of altered conditions of development may be followed by marking the artificially propagated fry on liberation, and then observing the characters of the adults on recapture. The integrity of populations may be tested by tagging young to adult fishes caught in nature, and tabulating the returns. So also may the spawning grounds and seasons of each unit be determined. This is a very important point, for racial segregation tends to be most intense during reproduction. Simple transference experiments, with or even without individual marking, will no doubt often be of real aid in experimental race analysis. The testing of the assumed hybrid nature of species-intermediates found in nature, by resources to such controlled matings as we are now making with American Centrarchidae and Poeciliidae (Hubbs and Hubbs, 1932a and 1932b), will no doubt become an important phase of experimental ichthyology.

The introduction of experiment into variation investigations, and the wide-spread use of statistics in genetic work, should bring the two fields somewhat closer, at least as far as methods of research are involved.

II

After this brief view of the present status of our knowledge of racial and individual variations, with a limited attempt at envisioning the near future, a brief abstract of some of the work already accomplished in this field is perhaps in order. The literature on variation in fishes will be chiefly discussed, but some mention may be made of similar work with other types of animals.

A considerable amount of attention has been given to the analysis of variations in the shells of mollusks. An outstanding contribution in this field is that of Adams (1915) who analyzed the remarkable downstream increase, in heaviness and spinosity, in the shells of the genus *Io*. Mr. Calvin Goodrich, a specialist on the fresh-water gastropods of North America, finds that in many species of related genera, the same variational trend is apparent. No one really knows, however, whether these great differences are actually genetic. Fresh-water mussels, similarly, tend to become more globose in larger waters (Ball, 1922), but again the observed differences may not really be racial. The excessive raciation undergone by the little land-snail *Partula,* in correlation with colony-isolation in tropical Pacific islands, is the subject of elaborate monographs by Crampton (1917, 1925, 1932). Variational studies of mollusks by European investigators are reviewed by Rensch (1930). A general paper of interest is Boycott's (1928).

Among crustaceans, the Cladocera show the most remarkable variations, both locally and seasonally. Many papers have been written on these points, but space forbids reference to them. The contributions of Wesenberg-Lund (1926, etc.) and Woltereck (1932, etc.) are perhaps the most important. The modification of marine relicts among higher Crustacea has been discussed by Ekman (1919) and others.

The most extensive variational analysis in insects is perhaps that of Tower (1918), whose spectacular results on the experimental evolution of the beetle *Leptinotarsa* have been looked on with much distrust. Perhaps if all the energy which has been spent in condemning Tower's work had been combined in a repetition of some of his experiments, biology in general and experimental systematics in particular would be farther advanced. Work on variation in insects (also in amphibians and reptiles) is reviewed by Rensch (1929).

Most of the variational studies with birds has been done as a mere accessory to subspecies discrimination,

and has been unsatisfactory from a statistical point of view. Some of this work, however, has proved for the purposes of taxonomy and distribution to be of fine character; witness such studies as that of Chapman (1926) on the birds of Ecuador. Perhaps the best race analysis of birds, from a bionomic standpoint, is Linsdale's recent (1928) study of variations of the fox sparrows of western North America. Rensch (1929), in his book on geographical variation in animals, naturally emphasized birds, as the author is an ornithologist.

Pioneer work on geographical variation, with particular emphasis on variational gradients correlated with climatic changes, was published by Allen (1871 to 1877), who worked with both birds and mammals. Much of the variation work done on warm-blooded vertebrates toward the end of the last century was based on Allen's foundation. The contributions by Miller (1909) on the differentiation of mouse deer in (supposedly) similar environments, and by Osgood (1909) on the subspecies of American mice of the genus *Peromyscus,* over an area of high climatic contrasts, may be mentioned. The outstanding investigations on the variation of mammals in recent years is the splendid work of Sumner (1924, 1926, 1932, etc.), also with *Peromyscus.* He has proved conclusively, among other points, that many of the fine distinctions used to distinguish subspecies in mice, and often correlated closely with climatic conditions, are genetically fixed. These *Peromyscus* investigations are being extended by Dr. Lee R. Dice (1932, and papers to follow).

Other work on variation in the higher vertebrates is referred to by Osborn (1927), who has given a general discussion of "speciation."

III

One of the first zoologists who gave evidence of critical interest in variation within fish species was Heincke, for as early as 1880 and 1883 he (on the latter date with Möbius) discussed the differences distinguishing the Baltic and North Sea races of certain fish species. In

this work Heincke observed that the differentiation of the brackish types of unrelated species had been parallel.

Several American zoologists worked on variation within fish species before the close of the nineteenth century. We may refer to Eigenmann's work (1895) on the geographical variations in number of anal rays in *"Leuciscus" balteatus;* to Moenkhaus's papers (1894, 1896, 1898) on the darters and to Rutter's study (1896) of *Gasterosteus.*

A great advance in the study of variation in fishes came with the appreciation of the importance of race analysis in fisheries biology. In fact, the real foundation of race investigations in fishes is the classic work of the pioneer Heincke (1898), on the herring of Europe. Many other investigators have since worked on this species, for example Johansen (1919, 1924), Lea (1919, etc.), Ehrenbaum (1929), Strubberg (1930), Schnakenbeck (1931) and others. Thompson (1917), Hubbs (1925), Fujita and Kokubo (1927) and Rounsefell (1930) have made similar investigations on the Pacific herring, and several Russian ichthyologists are now publishing racial studies of both forms. These authors have carried the analysis of herring races into significant detail, but many questions remain debatable.

European investigators have analyzed the variation in other species of commercial importance. The literature is large, but much of it has been reviewed by Redeke (1902, 1912, 1915). The work of Duncker (1900, 1913, etc.) on flatfishes is among the best, but this author's most elaborate variational study (1908) was based on a pipefish, *Siphonostoma.* Fage, to mention only one other, has studied variation in *Mullus* (1909) and in clupeoids (1911, 1920).

A splendid example of parallel raciation was found by Tåning (1918), in statistically comparing the several species of lantern fishes in the Mediterranean and the Atlantic. Ege (1930) obtained similar results in comparing the races of two species of *Paralepis* in the same region, as did Chabanaud (1929) with a sole.

That the remarkable homing habits and consequent

stock-isolation in the Pacific salmon is correlated with marked structural differentiation is suggested by the work of McGregor (1924). This investigation should be extended, because of its biological interest as well as its economic significance.

The most extensive and best known race investigations with fishes are those of Johannes Schmidt (1917 to 1930) on *Zoarces* and other fishes, continued also by Kirstine Smith (1921, 1922). Schmidt's results are too well known to require much review. The analysis of thousands of specimens of *Zoarces* showed that this relatively stationary fish exhibits marked local variations, which are inherited though slightly modifiable; that the variations are correlated with hydrographic conditions, but not perfectly so correlated; and that the variations show annual fluctuations. Schmidt also obtained valuable results with individual modifications and inheritance in *Lebistes* and *Salmo*. In a more recent work (1930), a masterly investigation on the variation of the cod in the North Atlantic showed so strikingly the correlation of structural characters with water temperatures that the author placed more emphasis on this correlation than previously. In his *Zoarces* investigation, Schmidt made a rather strained effort to minimize the relation of the environment to the racial characters.

In the work done in my laboratory (Hubbs, 1918 to 1929; Hubbs and Whitlock, 1929; Schultz, 1927, etc.), we have stressed geographic and climatic trends in variation; parallel raciation; the correlation between rate of development and meristic characters; the probably indirect, adaptive significance of most racial distinctions; the parallel but presumably unrelated racial and individual modifications, especially those determined by temperature acting during development; the great modification of characters by such factors as early parasitization and siltiness of water. We are continuing our investigations along these lines.

A large number of papers on variation in fishes are now being published each year by the Russian ichthyologists, unfortunately in the Russian language.

Interest is growing in the correlation between the variations exhibited by fishes and the physico-chemical environment in which they develop: witness such papers as these: d'Ancona (1927), Johansen (1928), Schnakenbeck (1931), Mottley (1931) and Parr (1932). Signs are not lacking that the study of variation in fishes and other animals is being rejuvenated into a live division of zoology. This study may well assume such a position, if it is prosecuted not as an end in itself, but as a tool to be used in solving problems in systematics, economic zoology, zoogeography and evolution.

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THE RESULTS OF TWENTY YEARS OF SELF-FERTILIZATION IN THE POND SNAIL *LYMNAEA COLUMELLA* SAY

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INTRODUCTION

As a contribution to the study of evolution, in 1911 an experiment was started to test out the effect of environment on the visible characters of an animal. The effect of heredity must be controlled as well as environmental factors. The pond snail *Lymnaea columella* was selected because the senior author had found that these hermaphroditic snails, when isolated, laid fertile eggs which were self-fertilized (Colton, 1912, 1918). This observatoin was confirmed by Crabb (1927). We can, therefore, establish a pure line in animals in the same sense that it can be done in many plants.

METHODS

A pure line established with *Lymnaea columella* makes this snail a standard animal. A standard animal is an animal easily available which, under controlled conditions, always reacts in the same way, and if in any way it should deviate from the average the magnitude of deviation is known. A short life cycle, abundant food, an easily controlled environment and a hereditary constitution so homogeneous that all the individuals practically have the same heredity—these are the specifications of a standard animal.

In December, 1911, our line was started, and since then the descendants of the original parent have been under controlled conditions. Notwithstanding the fact that for 93 generations the closest kind of inbreeding has been practised, each young snail has been isolated in a jar by

itself and being hermaphroditic has fertilized its own eggs (see Colton, 1912, 1918; Crabb, 1927), producing a race of gametic purity rarely attained in animals. Nevertheless, this race shows remarkable hardiness. Rarely does an egg once laid and kept under favorable conditions fail to hatch. Barring accidents, with plenty of food, water and air they live a year or two. Exactly how long has never been determined.

Lymnaea columella reacts readily to slight environmental changes, so a controlled environment becomes necessary. 500 cc of filtered pond water placed in a 4½″ by 5″ battery jar forms the standard medium into which is introduced a single snail. The jar containing the snail is kept at room temperature which, between October and April, ranges from 19° to 22° with a mean of about 21°. In the summer months a temperature approaching 30° accelerates the life cycle.

Chemical analyses of the medium show that the water in which the snail lives is practically without oxygen. Since the snail is air-breathing, this anaerobic condition offers but slight disadvantage to the animal, and since no green plants are present the small amount of oxygen absorbed by the surface of the water is completely absorbed cuticularly by the snail (see Colton, 1908; Walter, 1906).

For food, dried leaves of the Carolina poplar are introduced, one at a time. Small tender leaves are chosen at first, when the snails are small, and larger, tough leaves later. It is necessary to add but few leaves at a time and to add no leaves that appear green, for these seem to form a favorable medium for bacteria. The bacterial skums from such leaves keep the young snails from reaching the surface and cause them to drown. Dried leaves can be gathered in the autumn and kept indefinitely. Dried leaves certainly furnish all the materials for building protoplasm, supply necessary vitamins and furnish potential energy that the snail can release. It

may be interesting to mention that the pond snail is almost the only animal in our Pennsylvania ponds and streams which is adapted to converting the dead leaves that fall into the water into a form, snail meat, which will support crayfish, fish and water birds.

As the senior author pointed out some years ago (Colton, 1908), this snail has a true gizzard in which are small stones. These stones are necessary for the breaking up of plant tissue. Without stones the animal starves amid abundance. Therefore, it is necessary to introduce a pinch of soil into each jar.

The curve of growth of the standard snail (22nd self-fertilized generation) shows that after seventy days the growth is constant and the line becomes nearly straight. If the standard deviations from the means are plotted, the variation is seen to be much greater on the steep part of the curve than on the gentle slopes. All the snails do not have the same start in life from the same egg case, some hatching two days after the first, yet this lack of start is not enough to cause great variations after 21 days. A study of the curves of the individual snails show that they all tend to approach the mean after 70 days. Experiments with this snail should, therefore, run 70 days to procure the most accurate results. This agrees with the work of Bailey (1931).

The choice of the parent for each generation, as far as visible characters are concerned, is left to chance. No conscious selection was made. However, in most cases, the snail from a given egg case which first reached sexual maturity was selected as the parent of the new generation.

Considerable variation from the time of laying until hatching—from 8 to 12 days—is observed even under standard conditions. If sexual maturity is considered as the time of laying the first eggs, this is seen also to be very variable. In general, when a snail reaches the length of 10 mm it usually lays eggs, but the range ex-

tends from 7 mm to 12 mm. Forty days after hatching, the first eggs are usually laid. This interval is much longer in the autumn, often extending over three months, and is shortest in the spring, five weeks. The factor prolonging the life cycle in the autumn is neither food nor temperature and at the present time is unknown and uncontrolled. The same phenomena have been observed by the senior author in the white rat.

The race of *Lymnaea columella,* self-fertilized for 93 generations, has probably a greater gametic purity than any other animal which reproduces sexually. Inbreeding of the closest sort, self-fertilization, has not appreciably affected the viability of the race. The results accord directly with King (1918 a, b, c), Wright (see East and Jones) and others. That is, with a race containing no recessive defects, inbreeding of the closest sort is not harmful.

RESULTS

The present study comprises a comparison of snails of the 82nd to 85th self-fertilized generations with earlier generations as well as a comparison with "wild" snails

TABLE I

	Mean ratio	Coef. of variation of ratio	Mean index	Coef. of variation of index	No. snails
Original wild Parent, No. 1152	1.29		1.69		1
1st generation Parent, No. 1193	1.36		1.65		1
2nd generation Parent, No. 1220	.....		.....		1
3rd generation Parent, No. 1243	1.46		1.67		1
4th generation Parent, No. 1283	1.34		1.78		1
5th generation Parent, No. 1305	1.43		1.77		1
6th to 10th generation (1913)	1.400 ± .004	3.63 ± .170			99
11th to 15th generation (1914)	1.392 ± .003	3.92 ± .214			168
82nd to 85th generation (1930)	1.365 ± .004	4.24 ± .306	1.757 ± .007	5.74 ± .414	96
Wild Random Sample (1912)	1.375 ± .0018	4.39 ± .131			516
Wild raised under standard conditions (1930)	1.414 ± .003	3.46 ± .223	1.763 ± .007	6.18 ± .400	119
B line 6th to 10th generation (1913)	1.476 ± .004	3.35 ± .300			63

and with the progeny of "wild" snails raised under standard conditions. The data is presented in Table I.

Three measurements were made on most shells (Fig. 1): altitude AB, length of aperture BC, and, in some

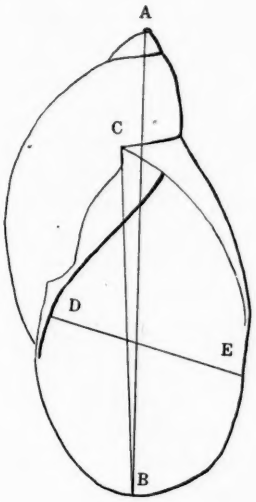

FIG. 1

cases, width of aperture DE. From these measurements two ratios were computed which together give a picture of the shape of the shell. The altitude divided by length of aperture will be called the "ratio" and the length of aperture divided by the breath of the aperture will be called the "index" (Colton, 1912).

The problem has been to distinguish significant differences if any between the 82nd and 85th generation and earlier generations and significant differences if any between the 82nd and 85th generations and the progeny of wild snails raised under the same conditions as the experimental line.

The significance of the difference is determined by the relation of the difference to its probable error.

If the difference is three times the probable error, then the chances are 4.5: 1 that the difference is significant and not due to chance (Davenport, 1904).

If the difference is ± 4E, then chances are 142: 1.
If the difference is ± 5E, then chances are 1,310: 1.
If the difference is ± 6E, then the chances are 19,200: 1.
If the difference is ± 7E, then the chances are 420,000: 1.
If the difference is ± 8E, then the chances are 17,000,000: 1.
If the difference is ± 9E, then the chances are about a billion to 1.

A study of Table I shows that the original parent snail of the line had a very short spire (ratio 1.29). On the other hand, the third generation parent had a particularly tall spire (ratio 1.46). The mean ratio of all snails of the sixth to tenth generation, 99 snails in all, was 1.40. Since then the spire seems to have progressively shortened, the mean ratio of the 82nd to 85th generation being 1.36. That this difference is too great to be a matter of chance is clearly indicated in Table II.

TABLE II
DIFFERENCES

Differences between different groups	Differences between means of ratio	Difference divided by PE	Diff. between coef. of var. of ratio	Diff. between means of index	Diff. between coef. of var. of index
82 to 85th less 6 to 10th	−.035 ± .007	5	.61 ± .350		
82 to 85th less 11 to 15th	−.027 ± .005	5	.32 ± .373		
82 to 85th less wild sample	+.010 ± .004	2.5	.15 ± .333		
82 to 85th less wild in captivity	−.049 ± .005	10	.78 ± .379	.006 ± .009	.44 ± .575
82 to 85th less 6–10 B line	−.111 ± .006	18	.98 ± .428		
6 to 10th A less 6 to 10 B	−.076 ± .006	12	.26 ± .345		

In order to observe the magnitude of direct environmental effects, a line called the B line was established. Progeny of snails of the fifth generation of the A line were raised in very shallow water, 30 to 50 cc in crystallizing dishes. The water was so shallow, about 4 to 6

mm, that the poplar leaves were often not covered by water but were merely damp and partly exposed to the air. The effect of the condition on the snail was a taller spire. Whereas, the ratio of the sixth to tenth generation of A line was $1.400 \pm .004$, and sixth to tenth of B line was $1.476 \pm .004$. Table II shows how significant the difference is. When descendants of this B line were placed under the conditions of the A line, the tall spire was not retained. We, therefore, had an example of the direct effect of environment which has no evolutionary value.

The shape of the aperture measured by the index seemed to be unaffected by the environment under the standard conditions. In every case computed, the differences recorded are less than three times the probable error.

One would expect the coefficient of variation of snails so closely inbred to be less than in the wild snails. No significant difference is visible. The differences between the means are less than three times the probable error in every case. Crabb (1927) has shown in *Lymnaea stagnalis* that self-fertilization is normal in *Lymnaea* and that although snails are frequently seen mating the chances of cross-fertilization are very remote.

Conclusions

(1) The spire of the standard line seems to have become progressively shorter after the sixth generation.

(2) The spire of the standard line is much shorter than the spire of the progeny of wild snails raised under standard conditions.

(3) Snails raised in shallow water have taller spires.

(4) The shape of the aperture seems unaffected by changes in the environment.

(5) One would expect the inbred lines to be less variable than the wild snails but this is not the case. No significant difference can be distinguished.

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TAXONOMIC DISTINCTIONS VIEWED IN THE LIGHT OF GENETICS

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By taxonomic distinctions I intend the morphological or physiological peculiarities by which animals belonging to one group may be distinguished from those belonging to any other coordinate group, whether or not these peculiarities are ones which have been recognized by systematic zoologists as diagnostic. By genetics I intend the whole body of accurate knowledge concerning the transmission of morphological or physiological peculiarities through successive generations, whether or not it has been found possible to explain these peculiarities in terms of Mendelian factors.

It is fairly obvious that any concrete discussion of this subject must be limited to the least inclusive taxonomic categories: subspecies, species or, at most, genera. Concerning the genetic status of the major subdivisions of the animal kingdom, one can, at present, only speculate.

It is not long since many, perhaps a large majority, of systematic zoologists rejected a Mendelian interpretation of the characters in which they were chiefly interested. It is likely that some of them still do so. Their attitude was quite natural and not altogether attributable to ignorance or perversity. Indeed, the prima facie case was all in their favor. Collectors and systematists had long been familiar with aberrant characters, for the most part marked peculiarities of coloration, which were known to be inherited in alternative fashion. Such aberrations were commonly not recognized in taxonomic nomenclature. They were sporadic in occurrence and had nothing to do with the characters which distinguished species. Indeed, some of them, such as albinism and melanism and various dilutions of the typical plumage or pelage color,

were known to crop out independently in widely different families or orders.

On the other hand, it was equally plain that the distinguishing characters of species or geographic races were not inherited in alternative fashion. The many natural hybrids which were known to naturalists displayed, for the most part, a mixed condition, intermediate in respect to some of the most salient characters.

Acceptance of the more modern view-point was made yet more difficult by the utterances of the earlier mutationists. There was formerly much talk of the origin of species by sudden saltations, although some, it is true, among those who employed such language had in mind smaller groups than the ordinary Linnaean species. However, the steps were in general supposed to be of sufficient magnitude to account for the wide-spread discontinuity in the organic world, and to avoid the long recognized difficulty concerning the "pre-useful stages" of useful characters.

The taxonomist, on his part, felt equally certain that most of his species had not arisen in any such abrupt way. And since "mutation," to him, meant just that kind of abrupt origin for his species and geographic races, he naturally and wisely rejected it. The wisdom of his course has been demonstrated by the later history of the mutation theory itself.

Few biologists need be reminded of the revisions here referred to, both in the conceptions regarding mutation and in the factorial scheme of inheritance. However, it may be well to review the situation briefly as a basis for further discussion.

Experimental breeding soon revealed the fact that discontinuous characters, showing an alternative type of inheritance, were not all large or conspicuous. Many were found to be insignificantly small, detectable, indeed, only by the most careful inspection.

It was further discovered that certain apparently simple character differences between organisms depended

upon the cumulative action of two or more pairs of genetic factors instead of one. Since these factors segregated independently of one another, in the formation of the germ-cells, all possible chance combinations occurred among the derivatives of a cross which involved a character of this sort. There might thus arise a graded series of values in the manifestation of the character in question. Mendelians were quick to recognize the theoretical importance of this discovery. They saw in it the probable interpretation of a large class of phenomena which had hitherto baffled them, and which had seemed to seriously limit the scope of the Mendelian laws of heredity. Reference of course is made to the numerous cases of blending inheritance, in which there is a more or less permanent mingling of the parental contributions, not only in the first but in later hybrid generations.

This hypothesis of "polymeric" or "multiple" factors is now generally accepted, at least as a fruitful working hypothesis. Indeed, there is at present, so far as I am aware, no definitely formulated rival hypothesis in the field. It should be remembered, however, that in the vast majority of cases this is still merely a hypothesis. In only a few instances has an actual factorial analysis been made. It is likewise of interest to recall here that the essential part of this hypothesis—the resolution of apparently blending inheritance into discrete units—was clearly stated by Francis Galton in 1889, though naturally without its Mendelian context.

To-day the geneticist no longer asks concerning any distinguishing character of an organism: Is it or is it not Mendelian in its hereditary behavior? He asks rather: Is it dependent upon a single pair of Mendelian factors, or does it belong to that far larger class of characters which are dependent upon the cumulative action of a number of such factors? So far as concerns the mammals and birds, at least, I think that we are already justified in assigning the great majority of taxonomic differences to this second category.

My own most immediate knowledge in this field is derived from a study of the species and geographic races of white-footed mice.[1] In this genus, the chief taxonomic characters, both of species and subspecies, relate to such features as total size, the proportionate dimensions of the tail, feet and ears, of the skull, and some other bones, the color of the pelage, the relative extent of the colored and uncolored areas of the hair, and the pigmentation of certain regions of the skin. These are all characters which may be subjected to quantitative treatment. In very few cases are species or races of the mice distinguished by the possession of a character of the all-or-nothing type, a character, that is, which is present in full measure in one, but totally lacking in another.

So far as investigated, the peculiarities of the various geographic races or subspecies are found to be wholly genetic. The differences between the mean values for any two races persist undiminished when the animals are reared in a common environment.

The individual differences within a subspecies, on the other hand, are partly genetic, partly phenotypic, whatever that last word may mean. In any case, they are only partly hereditary.[2] So far as they are genetic, these individual differences are of the same type as those which distinguish subspecies from one another. We have darker and paler geographic races, darker and paler individuals within each race; longer and shorter-tailed races, longer and shorter-tailed individuals, etc. It not infrequently happens that a racial difference can be expressed only by a comparison of the mean values of the character in question. The ranges of the individual

[1] Sumner, *Journal of Genetics*, Vol. xxiii, 1930, pp. 275–376; *Bibliographica Genetica*, ix, 1932, pp. 1–106. The latter gives a complete bibliography of the Peromyscus studies of the author and his collaborators.

[2] This difference between individual and racial variation is responsible for the fact that two "characters" may be found to vary together when a series of geographic races are compared, while little or no correlation between these same characters may be detected within any single local population.

values for the respective races overlap broadly. There seems to be little doubt that these geographic races have arisen through the accumulation of the same sort of factorial differences as are scattered at random throughout each race.

So far as I have been able to determine, all these quantitative differences between the subspecies of *Peromyscus* are of the polymeric (multifactor) type. Not one point of distinction has been encountered which gives evidence of depending upon a single factor difference or even upon a very small number of factors. An analysis of the various hybrid generations leads to the conclusion that the difference in coat color between such closely related forms as *Peromyscus polionotus albifrons* and *P. p. leucocephalus* must depend upon a minimum of five or six factor differences. As regards differences which relate to linear measurements, the factorial composition is so complex, or perhaps is so obscured by the occurrence of phenotypic differences, that there is little or no evidence of segregation in the second hybrid generation or in back-crosses.

It is important to record here the genetic behavior of one character which in advance might have seemed more likely to follow an alternative type of inheritance. I refer to the dark longitudinal stripe on the dorsal surface of the tail. *Peromyscus polionotus polionotus* possesses a stripe which extends through the entire length of the tail. In *P. p. leucocephalus* this stripe is entirely lacking. In crosses between these two races, this character showed as little indication of being determined by a single pair of factors as did those in respect to which the parent races differed only in degree. Partial stripes, of varying length and intensity, predominated in both F_1 and F_2 generations, with a considerably higher variability in the latter. Furthermore, these varying grades proved to be in part genetic.[3]

[3] This is not an adequate account of the situation, but will suffice for present purposes.

Another circumstance of interest with relation to the inheritance of pigmental characters in *Peromyscus* is the fact that these various characters were found to be in a high degree correlated. An analysis of results from hybridization experiments led me to the conclusion that this correlation was due to the diverse effects of the same genes rather than to linkage among different genes. It was equally evident, however, that these various color "characters" were determined in part by independent genes.

Thus, for one genus of mammals, at least, it seems certain that even the geographic subdivisions of a species do not arise as single mutational steps. And since closely similar phenomena of geographic variation are exhibited in many other genera of mammals and by multitudinous species of birds, it seems probable that the above statement may be generalized so as to include these two classes of vertebrates.[4]

However, in stressing the phenomena of geographic variation, I am not overlooking that other, quite different class of heritable variations already referred to, even though these have, for the most part, received no taxonomic recognition. Early in our *Peromyscus* studies, several marked color variations of the "mutant" type presented themselves, while hairless individuals cropped out in two independent descent lines. These aberrant forms proved to be, for the most part, one-factor recessives.

Such large-scale mutational changes are well known in other genera of mammals, as well as in birds, insects and

[4] It would not be safe, however, to extend this conclusion to all subdivisions of the animal kingdom. A quite different situation has been described by Goldschmidt (*Arch. für Entwickl.*, 101 Bd., 1924; 116 Bd., 1929) for the gypsy-moth (*Lymantria dispar*). The color differences shown by the numerous geographic races of this moth are believed by him to depend upon systems of multiple *allelomorphs*, rather than upon multiple factors. Yet other quite distinct types of geographic variation are those described by Dobzhansky (AMER. NAT., Mar.–Apr., 1933) for certain coleoptera, and by Crampton (Carnegie Inst. Publ. 228, 1916, and 410, 1932) for the land gastropods of some of the Pacific Islands. It would be difficult to reduce all of these, particularly the case of the gastropods, to a common basis.

some other major groups of animals. In not a few instances, the mutant forms have multiplied to such an extent as to constitute an appreciable fraction of a local population, interbreeding with the typical form, though frequently maintaining their distinctness through genetic segregation. Instances are known in which they have even superseded the typical form altogether in certain parts of its range. The much discussed cases of melanism in English moths, and various examples among the birds, are cases in point. Owing to the frequently discontinuous character of these changes, and their usual unrelatedness to any known environmental factor, they have often been contrasted with geographic variations as something fundamentally different. Thus Chapman contrasts "mutational" and "environmental" characters in birds, the former being held to be "qualitative," the latter merely "quantitative." He believes that both have played a prominent part in the evolution of this group.

It is likely, as already stated, that the genetic difference between these two classes of characters is merely one of complexity, however fundamentally they perhaps differ in their relation to environmental factors.

Thus far, my discussion of the genetic status of taxonomic groups has been restricted to subspecies, the least inclusive of the categories ordinarily recognized by systematic zoologists. The differences between subspecies and Linnaean species are, however, to a certain extent conventional, and the assignment of a given group of organisms to one or the other category is often arbitrary. According to a widely accepted definition, two groups of closely related animals are regarded as subspecies if they occupy distinct but commonly adjacent territory and if the gap between them is bridged by intermediate types. In the absence of these last, the two groups are commonly regarded as "full" species. It is obvious that the presence or absence of intergrading forms may be entirely a matter of historical accident, and may bear no relation

whatever to the extent or the nature of the differences between the two groups.

In general, specific differences are of greater magnitude than subspecific ones. Different species may and frequently do occupy the same territory and habitat, while subspecies rarely or never occupy exactly the same territory, at least during the breeding season.

Subspecies frequently occur in geographic sequence, displaying graded series in relation to color, size, or some other character. Oftentimes there is a direct correlation between the color and environmental characteristics of their range. Species much less frequently exhibit such relations.

For this last reason, it has sometimes been contended that geographic variation is a process quite distinct from species formation, that the former is something superimposed upon the latter. Along with numerous zoologists, I cannot regard this contention as tenable. However, the question which concerns us here is not the evolutionary but the genetic one, namely, whether there is any essential difference in the mode of hereditary transmission between characters which are regarded as specific and those which are regarded as subspecific.

So far as bodily manifestations go, we are concerned with the same type of differences in coloration and in the relative size of parts. This fact is, of course, not decisive as regards the inheritance of these differences. We must appeal to the results of experimental breeding.

Experiments in interspecific hybridization have been conducted by a considerable number of investigators, and upon animals belonging to several of the major subdivisions of the animal kingdom. Insects, fishes, birds and mammals have been subjected to such tests. While interspecific hybrids are largely infertile, there are many known cases of partial or complete fertility among them, even in a few instances where the parent species are commonly assigned to different genera.

The literature detailing these experiments is widely

scattered, and I know of no writer who has done us the service of bringing together the data and dealing with them comprehensively. In many cases, furthermore, the primary object of the investigator has been not so much to ascertain the genetic behavior of specific characters, as to determine how a particular mutant character introduced by one species would behave in a cross with another.

I have not, myself, had an opportunity to examine the matter with any great thoroughness. Certain preliminary conclusions may, however, be stated with some confidence. These are largely identical with those which have already been recorded for subspecies. So far as I am aware, no two species which have been hybridized have behaved as Mendelian alternates. Several or many factors have been involved in all cases. Furthermore, most of the distinguishable elements which constitute a given specific complex of characters are likewise inherited in polymeric fashion. While animals of the F_2 and back-cross generations may display color markings or other features which are primarily derived from one or the other parent race, these seldom occur in proportions which indicate a one-factor basis, and they are commonly imperfect in their manifestation.

Consider also the fact that two species usually differ from one another to some extent in total size, or at least in the relative size of the various parts of the body. With very few exceptions, these so-called quantitative characters have been found to display a blending type of inheritance in crosses of all sorts. The presumption, accordingly, is that most species differ by considerable numbers of these polymeric size factors.

It has long been evident that the earlier notions of the mutationists regarding the origin of species by abrupt large-scale germinal transformations do not apply to such animal species as have been successfully hybridized. Any two of these which have been tested have been found to differ by a great number of genetic factors. If each

of these factorial differences is to be attributed to an independent gene mutation, the number of these mutations concerned in the production of even a single geographic race must have been considerable.

However, I think it misleading, in most cases, to speak of the formation either of a race or a species through the accumulation of successively appearing mutations in a given direction. If selection is at work, it probably favors individuals which differ from the average of the population by large numbers of factorial differences. Few or none of these last may have been the result of recently occurring mutations. There would seem to be much in favor of the contention that the crossing of genetically different individuals is the immediate source of most of the variation upon which selection acts.

The ultimate source of the variation is, of course, the mechanism, still unknown, which produces the single genetic changes. That these changes are utterly random, prior to selection, is doubtless the present belief of most geneticists. That the environment may, on the contrary, have a well- marked directive influence upon them is the belief of many students of distribution. The frequent parallelism between structural or pigmental gradients and climatic gradients furnishes one basis for this belief.

It may be wondered why I have omitted all reference to certain other types of abrupt genetic changes besides those which are commonly attributed to the modifications of single genes. Cases are well known in which the number of chromosomes in the nucleus has been increased through failure of the mechanism of division, giving rise to fertile mutant types showing characteristic departures from the parent form. Allied to these are cases of hybridization between species having widely different numbers of chromosomes, or having sets of chromosomes which are only partly homologous in the two species. In a few of these cases, appropriate readjustments of the respective chromosome sets have taken place, resulting in permanent homozygous hybrids carrying the entire

heritage of both species, or in some instances, only part of this.

If I were a botanist, I should doubtless have laid much more stress upon these last types of genetic transformation. Normal polyploidy appears to be far commoner among plants than animals, while interspecific hybrids seem to figure much more prominently in the flora of a country than in its fauna.

To what extent species formation among plants has resulted from such happenings, I am quite incompetent to state. As regards animals, some experimentally produced hybrids have been reported for species of Lepidoptera having in part non-homologous chromosomes. None of these, it is true, have quite fulfilled the requirements for a permanent, stable hybrid. But if such a cross should chance to come about under natural conditions, and if any of the derivatives proved to be reasonably viable and fertile, and happened to be isolated from both parent stocks, we might have an instance of the abrupt origin of a new species differing in a large number of genes from any other known species. While this possibility must be kept in mind, we are certainly not justified at present in looking upon interspecific hybridization as an important immediate source of new species among animals. I speak of *immediate* source, since it seems probable that hybridization in the broad sense, chiefly between minor races, but also perhaps between species, is an important source of the variability upon which selection acts.

Passing to the genetic status of groups more inclusive than species, we have very little positive information. In several cases, however, fertile hybrids have been obtained from crosses between distinct genera. This fact is not as impressive as it might be, owing to the lack of any objective standard according to which a given degree of difference may be rated as generic rather than specific. The extent of taxonomic "splitting" depends largely

upon the intensity with which a particular group has been exploited by systematists.

One of these intergeneric crosses is that between the fishes *Platypoecilus maculatus* and *Xiphophorus helleri*, which has been studied by Gerschler, Bellamy and others.[5] In this case, a partial segregation of certain of the parental characteristics was evident. It is interesting that one clear-cut feature which is regarded as a generic character of *Xiphophorus* not only gave unmistakable evidences of segregation, but seemed to depend upon a very small number of differential factors.[6] The character in question is the so-called "sword" of the male, a prolongation of the ventral rays of the caudal fin.

The other intergeneric cross here referred to is that between the mallard and pin-tail ducks, which has been described by Phillips.[7] Here segregation is to be inferred merely from the greater range of variability in the F_2 generation as compared with the F_1.

In neither of the foregoing cases have the relations between the two sets of chromosomes been determined.

To raise the question of the genetic status of the higher taxonomic divisions of the animal kingdom—families, orders, classes and phyla—appears to be rather futile. It is true that crosses between different families and even different orders of fishes have been perpetrated,[8] but the resulting zygotes have never arrived at maturity. They have undergone incomplete development, ranging from the earlier cleavage stages to larvae which have hatched and lived for several weeks. Such experiments, however interesting from the standpoint of developmental physiology, are not likely to reveal the genetic differences between animals so widely unlike.

In general, it is obvious that the major features of

[5] Gerschler, *Zeitschr. für indukt. Abst. und Vererb.*, Bd. 12, 1914; Bellamy, *Genetics*, vol. 9, 1924.

[6] Gerschler estimates that there are three of these.

[7] *Jour. Exper. Zool.*, vol. 18, 1915; *Genetics*, vol. 6, 1921.

[8] Moenkhaus, *Proc. Indiana Acad. Sci.*, 1910. Newman, *Jour. Exp. Zool.*, vol. 18, 1915. Similar crosses have been effected among the echinoderms.

animal organization are, by their very nature, excluded from investigation along Mendelian lines. However certain one may be of the factorial basis of the wings, the eyes or the nervous system of an insect, he is forever barred from putting his theory to a test. This is because it is quite unlikely that a successful cross can ever be effected between an insect and a type of organism which lacks the entire genetic basis for any of these structures.

It is hardly necessary to point out that the occurrence of eyeless or wingless mutant forms which behave as simple recessives to the normal throw no light whatever upon the genetic basis of these structures. The "normal allelomorph" of the gene "eyeless" can hardly be credited with the capacity for forming a pair of eyes. No more, indeed, than the physician who averts the death of a patient can be credited with giving the patient life. That a single and extremely simple alteration may effect a radical change in an object, even to its complete annihilation, proves nothing as to the degree of complexity of the object itself or of the processes necessary to bring it into existence. The claim that various important features of bodily structure have already been brought into conformity with the Mendelian scheme of inheritance is justified only in this very restricted sense.

A steadily increasing array of mutant genes is being revealed, some of which are capable of bringing about profound changes of bodily structure. Opposed to these is the shadowy array of "normal allelomorphs," concerning which all we know in most cases is that they prevent certain specific modifications of the normal type. To what extent and in what manner they may be said to determine the fundamental organization of the animal is a problem for the future to solve. Until that time the question of the genetic status of the larger taxonomic categories will be largely meaningless.

MUTATION AND ADAPTATION AS COMPONENT PARTS OF A UNIVERSAL PRINCIPLE: IV. THE BEHAVIOR OF ORGANIZED UNITS

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THE discussion of this principle of rhythm or periodicity has up to this point been purely descriptive. We have made no attempt to define the underlying unifying principle that must exist in all of these "autocatalyzed" systems. Nor has any attempt been made to determine the fate of these systems. We have simply observed their behavior and found it to correspond to a certain "pattern." The "pattern" being the same, it seems possible to arrive at some general considerations concerning the manner in which it is traced. It is therefore proposed in this section to view these various autocatalyzed processes as a whole in order to see what we can find common to them all.

It is perhaps inconceivable that so many phenomena should follow a law which qualitatively, at least, is so simple. On the other hand, the search for a unifying principle is intuitive and universal. But when we come to consider what these various phenomena have in common the problem ceases to be simple. We must ask ourselves: What can electrons, atoms, molecules, colloids, bacteria, cells, organisms, growth, societies and many others that can not be suggested by one word, have in common? The simplest thing that the writer has thought of is that many of them may be regarded as "populations" of units, of various sorts.

Let us at first, therefore, restrict our consideration to that portion of them that can readily be considered to be "populations." If we think of them in this way, then it

may be possible that certain principles of behavior will be found to be common to all of them.

It is of course evident that the curves that we are discussing arise from a statistical consideration of the phenomena in question. Moreover, it is obvious that statistical data of this sort can only be interpreted by changing the view-point to a dynamic one. In doing this we leave the certainty of the statistical method behind. Now it happens that the only "populations" of which we know the dynamics reasonably well are the simple molecular "populations" known as gases and liquids; the simpler phenomena of the kinetic theory of gases are well known. We can, therefore, do no better than to consider our problem in this way. Our view-point, however, will be to consider the behavior of the individual molecule instead of the population as a whole.

We think of the molecules of a gas as possessed of free movement restricted to a certain range of velocities characteristic of the substance in question, and restricted in three-dimensional space only by the nature of the boundaries present. The range of velocity of motion is determined by the fact that each particle contains energy in excess of a certain minimum amount required for this state. This probably differs widely for certain individual molecules, but in general lies rather close to a statistical average. This energy content is comparable to the "vitality" of a higher organism and if measured for all the molecules of a given gas could undoubtedly be plotted and would yield a typical probability diagram. So that our gas, which we normally think of as a homogeneous state of matter, is so only when considered in a gross way.

If, as in cooling, we diminish the energy content of a gas below a certain amount, characteristic for each species of gas, and dependent upon the pressure upon the system, then some of our molecules suddenly surrender considerable amounts of energy, without any further change in temperature and adopt a new plane of exis-

tence. We now have a mixed "population" of gaseous molecules and liquid molecules; a population composed of "rich" and "poor." Just as in human society the rich and poor exchange places in the course of time, so the "rich" and "poor" gas molecules are exchanging places. Some of the "liquid" molecules obtain enough energy somehow to enter the gaseous state, while a corresponding number of "gas" molecules fall into poverty and enter the liquid state.

The situation that we are describing came as a relief of tension, and the fact that such an exchange between the two "strata" of the "gaseous society" occurs that resembles so closely what occurs in human society is not to be taken as an indication that a state of tension or serious "dissatisfaction" exists in the liquid portion of our system, but this is of course true only so long as the pressure on the system remains constant.

We have perhaps said enough to indicate that there is some kind of a very real struggle for existence among molecules for the possession of energy, if we could dramatize a process that takes place on so small a scale. The struggle may be as real as that of a litter of nine pigs for the teats of the sow when only eight of these exist.

To return to our gas. If the removal of heat continues more relief is obtained—more liquid is formed. With persistent removal of heat liquefaction is progressive. It is dependent on the rate at which heat is removed and the curve shows nothing of the rate at which this occurs —nothing of the degree of intensity of this internal drama. The curve merely shows that no further change in temperature occurs until either all the gas has become liquefied or all the liquid form present has been restored to its gaseous state. In this intermediate condition it is completely submissive or receptive, when considered as a "population" or as a whole, to the energy changes of the environment. Its yielding is definitely circumscribed by its own innate nature and capacities. The exact nature of its behavior under such stresses of "life," because we

are really discussing the "life-history" of these substances, is a "species" characteristic and could be used and is used in the identification of species of matter.

The gas having been liquefied, we find that in the liquid state the molecules are also characterized by an energy content restricted to a definite range. Moreover, in this form the substance has lost its power of three-dimensional expansion—it is now restricted to two—although it retains its relations to the gaseous state in the form of a "skeleton organization" known as the vapor pressure. Without attempting to describe it in detail, it is really a more versatile and complex system than it was in the gaseous state. It has more "organization"—in short, more properties.

It is interesting to recall at this point that similar "transition phenomena" exist between the liquid and the solid states, in which the addition or withdrawal of energy simply increases or decreases the amount of solid present without changing the temperature. It represents another zone of mutation.

However clearly we are able to picture the properties of gases, liquids and solids, developed above, the attempt to apply such pictures to the general group of "populations" under consideration encounters difficulties. The changes in the state of matter that we discussed were due to the addition or withdrawal of energy in the form of heat, under certain conditions. Certainly, if the analogy holds for all these various systems the transition through the mutation stage, *i.e.*, the changes in the submissive state, must be due to some variable which can be looked upon in the same way. In the case of physical growth any significant variation in the essential food-supply, such as carbohydrate, fat, protein, air, water and a considerable variety of accessory food substances, is comparable either individually or together to the heat energy in the molecular systems. But the analogy goes much farther than this. Both in the inorganic and organic systems the individuals exist in two states, *i.e.*, a resistant state, in which adaptation only occurs, and a

receptive or submissive state, in which mutations take place. So that although the population as a whole may be growing rapidly yet the individuals in it exist in two states.

So far as organic growth is concerned the resistant individuals are often called adults, while the submissive ones are the young of the species. In this respect the individuals of the population are comparable with the individual molecules of the gas or liquid in the so-called critical zone. Of course it must be understood that such a description does not cover all the "active" or mutational states of molecules any more than the factors mentioned control all the corresponding states of the organisms. It does, however, cover some of the most obvious and simplest factors involved in both cases.

This receptive state in inorganic systems and in organic ones is a most interesting and mystifying one. When we see it in the organism we consider it to be due to effects of one kind or another that resemble the phenomena of chemical catalysis, but this phrase carries no definite connotations as to just what happens. In the case of simple systems like gases, liquids and solids, the receptive state, so far as change of state is concerned, is brought on by the addition or removal of heat, but we are quite in the dark as to the exact effects of such transfers on the molecule itself. So far as the other mutational or reactive states are concerned we know still less.

However much we may lack of detailed knowledge, these systems as a group appear to have two traits in common.

(1) The systems exist in an energy field that is of a higher or lower potential with respect to the units of the "population" under consideration, *i.e.*, the energy-receiving or dispensing system under discussion.

(2) The receiving-dispensing system exists in two states. In one of them there is a free exchange of energy with the environment, *i.e.*, the system is responsive. In the other the exchange is restricted—the system is

resistant. The former is the mutational state; the latter the adaptive state.

The first of these propositions will be discussed more fully in what follows and is perhaps the more obvious of the two.

The second may be discussed briefly at this point. In the first section of this essay the views of DeVries on mutation were briefly reviewed and it was clear that for him organisms exist in two states, *i.e.,* in the states of adaptation and of mutation. The first of these corresponds to the resistant state; the second to the responsive state. Moreover, we gave an account of how DeVries was able to obtain the responsive state with certain plants. In other parts of this essay the parallel existence of these two states was traced in a number of examples and it is now evident that the parallelism is universal. Not only do molecules in the gaseous, liquid or solid state exist in these two states, but all higher states derived by suitable processes of organization, either connected with life or not, as the case may be, exist in resistant and responsive states. In the case of a gas the responsive state is technically called the critical zone, in which its molecules make leapwise adjustment to the potential difference existing between its own inner states and the external environment. In the case of protoplasm the responsive state is more diversified and exists in many forms, depending upon the scale of our momentary interest. For the species the responsive condition is found in the germinal stage of its development. For the individual it is in general greatest during youth or early stages of development. The individual organs, tissues and probably the individual cells themselves in their normal course of growth and differentiation pass through responsive states into adult resistant states. In short, as we have already seen, the state of an organism is not a steady state expressible by its food intake, energy output or in any other terms of this sort. There

are internal and periodic mutations, which are not fully predictable in man, even when the nature and strength of the environmental stimuli are known.

In lower animals something has been done to measure these periodic mutations, and in general the effort is more effective than in man. Thus the mutations of a worker bee under controlled conditions are fairly well known, as reflected by its changes in activity. The internal mutations of a rat have been investigated under the conception of internal drives. The activities of such a rat appear to have a definite relationship to certain internal physiological changes. These states seem to depend on various appetites, related to food and sex cycles, and circumstances associated with them. The results on the one hand indicate how little is known about these departures from a steady state, and, on the other hand, they indicate how data may some time be obtained for a more adequate understanding of the changes involved.

Having visualized these systems as "populations" and having pointed out some simple relationships that come out of such a view we may drop this aspect of the subject and attempt to develop some other general considerations. The picture of resistant and of responsive states that we developed leads us back to the autocatalysis picture that we borrowed from chemistry. Catalysis implies the change from a resistant state to a responsive state, and autocatalysis implies that something capable of producing this effect may be formed within the system under observation. These changes are fully as mystifying in chemistry as they are in the more complex systems in which we found a behavior corresponding to autocatalysis. However, here again we can develop some illuminating general considerations.

(1) A comparative view of the various autocatalyses mentioned in the preceding sections reveals the fact that the longer the duration of the autocatalyzed process the more complex the state of organization that must be developed in order that it may come into existence and the

higher the process lies in the realm of evolutionary development. For instance, the development of a new civilization depends upon the successful realization of such an enormous number of subsidiary autocatalyzed processes that we can marvel that it is ever achieved in fact. Viewed in this way, we do not wonder that evolution is so slow but rather that it has ever occurred at all. Moreover, it is impossible to conceive that the process is purely a matter of chance. Where there are so many component forces there must be a prevailing force in order that a given result may be realized. In fundamental respects the "energy" field to which the "organization" owes its existence is a directing force. But we have a sense that there is something more—something hidden—involved. The hidden thing is the receiving system and part of our difficulty arises from the fact that we ourselves are complex receiving systems. Being ourselves components of these autocatalyzed systems, through many intricate steps of organization, from the atom on up to the integration of our organs and bodily functions through the master organ—the brain—and then as individuals into social groups of gradually increasing dimensions, we have an intuitive sense that the process is or ought to be moving in a direction that we recognize as upward.

Some will consider this to be a fatalistic view-point. In some respects it is. For instance, in northern climates it formerly seemed inevitable that some children should suffer from rickets, but now that vitamin D is known, the rôle of sunshine in its activation, and its occurrence in foods, it is no longer inevitable that so many children, if intelligently cared for, should suffer from this disease. Vitamin D is an essential component of the "energy" field of normal nutrition. We could enumerate many other factors in this field. Other "fields" are built up in a similar complex manner, and it is only the unknown and uncontrolled factors that give the fatalistic trend to our thought.

(2) Another general characteristic of these systems that we recognize is that there is a "stepping-up" of the wave-length of the autocatalyzed cycles. We saw something of this in our consideration of the "spectrum of life." The small rhythmic movements present in all matter constitute a basic rhythmic system of pulsations that pervades all the universe, and these movements build themselves up moment by moment, and step by step, from one "overtone" to the other until we find ourselves speechless and unable to find words to express our impressions of the whole. It is another case of standing beside the Grand Canyon of the Colorado and recalling that this and many other scenic marvels of nature are but late chapters in a story relating to "Little drops of water, little grains of sand." The unified view of this "spectrum of life" does not give us a sense of futility. Instead of this we find ourselves possessed with a feeling that we are after all essential agencies in the forging out of some great creative climax. The "stepping up" involved here differs, however, from the formation of the Grand Canyon or of a mountain chain because it is a "stepping-up" of an energy or power. It resembles the earthquakes by which the mountains are formed. This "stepping-up" can be more clearly visualized if we remember that one insane man "marking-time" with absolute precision on the middle of a suspension bridge could destroy in a short time what it would require thousands of men years to restore. The "stepping-up" of the rhythmical contractions of that man's muscles would develop an increasing vibration in the structure that would lead to its destruction. The reverse also holds true, but it is difficult to think of so dramatic an example in the physical realm. In the intellectual realm it is easy to recall instances in which the efforts of a single man have in the end achieved so great a constructive result that it would take thousands of men years to destroy his achievement.

(3) This feeling of being an important individual con-

stituent in the working out of the unified plan at its higher levels suggests that the whole series of autocatalyzed processes is quantized, just as the lower portion of the series, having to do with electronic activities, most certainly is. In fact, having hit upon the notion it is really much more obvious at the higher levels. Moreover, the fact that change can never come from haphazard variations in response is clearly evident at the higher levels. We can illustrate this by visualizing the behavior of an army in comparison with that of an equal number of men taken at random, who must be considered as constituting a crowd. The individuals of the army have been trained to react in definite purposeful ways in response to stimuli. The individuals have been synthesized to behave as an organic unit. In the crowd the reactions are individual and therefore it is not an organic unit, and because of the haphazard quality of the response it represents chaos in comparison with the disciplined unity of the army. The crowd has no courage because it has no unity, whereas the courage of the individuals of an army is "stepped-up" until it becomes greater than the sum of that of its individuals; it is quantized and becomes an impressive military morale. Each man in the army is conscious of his own individual feelings and senses that of the military "organism" of which he is a part, as well. When he loses his sense of well-being in the "organism" of which he is a part, he is distressed in his own being. This "esprit de corps" is a form of energy, just as heat or light, and is transmitted and absorbed in much the same way, although it can not be measured by the same instruments.

It is important to note that the source of this "esprit de corps," this peculiar energy, is the officers and above all the commanding general. History records the fact that great generals are highly endowed individuals. In this case we have a perfect autocatalyzed system. The general and his staff officers develop within the recruits

by disciplinary training and personal example the qualities that they require.

By extension it becomes evident that human progress is a similarly quantized process, dependent upon the efforts of a few superlatively endowed individuals, multiplied by the efforts of a lot of "buck privates." It is for this reason that movement either forward or backwards is so hard to inaugurate. Moreover, such leaders must be endowed with a rare kind of patience and inexhaustible stores of energy or they will be overtaken with impatience and fatigue and cry out with the impatient and tired sages of ancient times: "Vanity, vanity, all is vanity, saith the preacher." The technique of true leadership has no place for an alternation of assertion and negation. Human progress can not be secured by an alternate application of heat and cold. In this respect it is like a chemical reaction—a fire.

(4) In a recent paper the writer has investigated certain problems connected with the behavior of organized units under the general title, "Chemical Atoms and Superatoms."[1] We are now prepared to apply and extend the picture developed there and to see that the similarity of behavior in all these systems is related to a change in scale. When a given phenomenon is viewed in such a way that it can be considered statistically, as the behavior of a "population" of a suitable scale, then its response to an environmental change, suitably conceived, will appear to conform to the form of the autocatalytic curve. However, in order that the change may occur in this way, a certain responsive relation must exist between the "population" and its environment conceived as a suitable form of energy. This, as was stated in the essay referred to, is the integrated relation between the survival of the fittest and the fitness of the environment, pointed out by L. J. Henderson as holding for organic evolution.

The reader who has followed so far will have appreci-

[1] *The Scientific Monthly*, 31: 350–360, 1930.

ated by this time the words of explanation and apology with which the first section of this long essay were introduced. We have in our fingers a few threads, some ravelings, from a great tapestry of nature. Something is suggested of the heroic qualities of the design, but we have not seen it clearly. We are in somewhat the situation of Dobereiner in 1839, when he spoke of the "triads" of the chemical elements, or of J. A. R. Newlands in 1866, who saw "octaves" of chemical elements. Shortly after this, through D. Mendeleeff and L. Meyer, these rude beginnings became the Periodic Law of the Chemical Elements. But it was nearly a hundred years after Dobereiner's gropings until these generalizations were perfected by Moseley. Disregarding the question as to whether the work of his predecessors was of any immediate help to Moseley, who undoubtedly was a peculiarly luminous genius, there is no doubt that the contemporaries and successors of Dobereiner, Newlands, Mendeleeff and Meyer benefited by even the obscure vision of this great generalization that they were able successively to supply to them. It is in the belief of the truth of this last statement that the writer has ventured to present this obscure and blurred picture of a great universal principle of nature. As a statement and exposition of the principle in question it may be set alongside of Dobereiner's "triads," as a rudimentary groping for light.

THE GENETICS SOCIETY OF AMERICA

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II

32. *Twins in the Jimson Weed, Datura stramonium:* SOPHIE SATINA, A. F. BLAKESLEE and A. G. AVERY, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. Seeds of the jimson weed germinate well. Occasionally as high as 101 per cent. germination has been recorded from counted seeds. In some of these cases the appearance of more seedlings than there were seeds planted may be explained by the fact that some of the seeds contained twin embryos. Twin seedlings have in fact been found coming out of a single seed coat, although most twins shed their coats before emerging from the soil, as is true also of single seedlings. In one case in which the parent was hybrid for a yellow type, it could be shown that we were not dealing with "identical twins," since one twin was yellow and the other twin was normal. Cytological study of ovule formation in the jimson weed shows that twins arise side by side within a single ovule from two distinct cells which have the reduced number of chromosomes. Twins in the jimson weed, therefore, arise like other sexually formed embryos and hence may be unlike in their hereditary constitution. They are thus unlike the extra embryos regularly found in grapefruit and other citrus seeds which are produced non-sexually by buds from somatic tissue of the ovule.

33. *Results Obtained from Spliced Styles:* J. T. BUCHHOLZ, C. C. DOAK and A. F. BLAKESLEE, University of Illinois. Continuation of experiments on spliced styles (*Bull. Torrey Bot. Club,* 59: 108–118) has yielded plants homozygous for a gene (sl_2), giving "slow" pollen-tube growth. An example of pollen tube distribution from plant 300703(29) heterozygous for this gene was given in the above. Control planting from selfs heterozygous for sl_2 gave only 3 per cent. homozygotes, while results from spliced styles gave 40.5 per cent. homozygotes (in total of 163 plants from 7 pedigree samples). The homozygotes are easily recognized, weak stunted plants with leaves rough and less deeply lobed than normal plants, coming from stems which do not fork in the manner characteristic for Datura. Flowers are seldom produced in the garden, their pollen is poorly developed or entirely absent, probably due to lack of proper nutrition of

the small anthers. Seeds have been obtained from greenhouse plants when pollen of plants homozygous for various genes and prime types was used. Important results of style splicing in this case indicate that the proportions of homozygotes may be increased many fold; giving 40.5 per cent. homozygotes, where 50 per cent. would be the theoretical expectation if the separation of normal and slow pollen tubes were perfect and the viability of homozygotes normal.

34. *Types of Mutations Affecting Pollen Grains which have been Induced by X-raying and Aging Seeds of Datura:* J. L. CARTLEDGE and A. F. BLAKESLEE, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. Abortion of definite proportions of the pollen grains of Datura may be due to (1) chromosomal mutations (chiefly rearrangements of chromosome segments by interchange), (2) genes for pollen abortion acting on the haploid pollen grains, and (3) recessive genes acting through the diploid parent. Although relatively rare in nature, both chromosomal and gene mutations causing pollen abortion have been obtained in large numbers from seeds treated with heavy x-ray dosages and from seeds which were aged by storage at room temperature for periods up to ten years. A majority of the plants grown from the most effectively x-rayed seeds have one or more pollen abortion mutations, usually in sectors of the plants. More than 8 per cent. of the plants grown from seven to ten year old seeds showed similar pollen abortion mutations. Plants from younger seeds gave progressively diminishing proportions of these mutations. We are able, in most cases, to distinguish between the chromosomal and gene mutations affecting the pollen by the structural appearance of the grains. The chromosomal mutations give uniformly small, empty, shriveled, aborted grains. Pollen abortion genes produce grains of subnormal sizes with variously abnormal contents. Pollen abortion furnishes a convenient and delicate index to mutation rate, and both chromosomal and gene types are useful and efficient in linkage experiments.

35. *Mutations and Somatic Variations Induced by High Temperature in Drosophila:* H. H. PLOUGH and PHILIP T. IVES, Amherst College. The completion of our most extensive experiment using the Goldschmidt-Jollos method of subjecting larvae of *Drosophila melanogaster* to a 30-hour exposure to a temperature of 36.5° C., gives us adequate data on which to base a final report of this work. In all our latest tests we have matings which reveal

X-linked lethals, visible recessive and dominant mutations. In a total of 186,000 flies from treated parents we have found and located 44 mutations, while the same attention to 73,000 control flies has given us 3 mutations. There are thus 5 times as many in the heated lines, and the difference is more than 6 times its probable error. Of the 44 mutations, 13 were X-lethals, 17 visible recessives and 14 dominants.

Both lines were carried at least 5 generations. Classification of the heat-induced mutations by generations shows that 28 could have been produced at the time of exposure, while 15 (35 per cent.) must have occurred later. Thus some temperature effect must have been carried over which increased the mutation rate for at least two later generations. This is unlike the results of x-ray treatment. Since all variations observed in the cultures were isolated, mated, and their offspring followed for at least two generations, accurate records are available for those which turned out to be non-genetic. Excluding the modifications in the generation actually heated, the data show that such somatic variations (somatic mutations) are 3 times as numerous among offspring of heated females, but no more frequent than the controls when the only heated parent was the male. Mutations on the contrary occur about as frequently among the offspring of either sex. This apparently disproves the idea urged by Jollos that induced somatic variations (Dauermodifikationen) subsequently become genetic (Mutations).

36. *Crossing-Over and Segregation in Somatic Cells of Drosophila melanogaster:* CURT STERN, University of Rochester. In special strains flies appear with morphologically aberrant regions (*e.g.*, "yellow" on an otherwise not-yellow individual). Such regions are produced by elimination of a chromosome (Bridges, Stern). A special gene (*e.g.*, Minute-n) in the eliminated chromosome itself was believed to be the cause of the elimination. New experiments indicate (as yet mainly for the X-chromosomes): (1) The elimination is not directly caused by the special gene acting on its chromosome. For: (a) elimination of the X-chromosome is brought about by Minute-genes in autosomes. (b) A gene in the X-chromosome causing its elimination ceases to produce this effect if it suppressed by a gene in an autosome. (2) Mosaic spots are the product of somatic segregation of a chromosomal pair (a $y^{+}/^{+}$. sn fly will show a yellow and a singed

area next to each other—twin spots! (3) In races without twin —but with single spots—one daughter cell from the somatic segregation division dies on account of having a lethal segregation product (a $^{+}$. Mn/y .$^{+}$ fly produces a Mn and a y cell; the first dies, the second gives rise to a yellow spot). (4) The somatic segregation follows somatic crossing-over. (5) This crossing-over occurs at a four-strand stage between two strands only. (6) In most cases the crossing-over occurs next to the spindle-fiber locus. (7) Somatic segregation leads sometimes to cells carrying two X-chromosomes (nearly identical: one cross-over and one non-cross-over) or to somatic reduction producing haplo-X (male) cells.

37. *Gene Deficiencies as Cell Lethals in Drosophila melanogaster:* M. DEMEREC, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. Among minute females appearing in crosses with blond translocation the incomplete X-chromosome is frequently eliminated during somatic divisions, leaving patches of tissue which can readily be identified if a marker (yellow or singed) is introduced into the other X-chromosome (Stern). Also on *y/sn* females, having autosomal minute, twin spots frequently appear with adjacent yellow and singed areas (Stern). If a gene deficiency or a short region deficiency is introduced into the whole chromosome of blond-minute females the elimination patches are either entirely absent or are few in number (somatic cross-overs) when compared with the sister females not possessing such deficiency. If a gene deficiency is introduced into one of the X-chromosomes of an autosomal minute female only single spots appear where twin spots are expected. This indicates that patches of tissues containing a deficiency can not exist even when surrounded by normal tissue. Since single cell spots can readily be detected in this material, their absence indicates that even single cells deficient for a certain locus are unable to exist. So far tests were made with seventeen single gene or short region deficiencies of independent origin and involving seven first-rank loci scattered through the whole length of the X-chromosome. In every case the deficiency tested had a cell lethal effect. This shows that the presence of certain genes (loci) is essential for the vital functions of a cell and suggests that the majority of loci may be of such type.

38. *The Effects of Alpha Radiation from Polonium in Produc-*

ing Somatic Modifications in Drosophila: FRANK BLAIR HANSON and FLORENCE HEYS, Washington University, Saint Louis. The biological effects of other radiation than beta and gamma have been a study of constantly increasing interest in the past few years. Ultra-violet, ultrasonic and electrostatic radiation and alpha radiation have been employed. Adults developing from eggs and larvae of Drosophila which have been exposed to alpha radiation from polonium show marked somatic modifications. These include abnormalities such as twisted head and thorax, half-thorax, gynandromorphs, malformations of the abdomen, reduplicated appendages, facet deficiencies and eyeless forms, and some changes closely resembling true mutations, *i.e.*, outstretched, strap, etc. All these were purely somatic, as genetic tests carrying the descendants through three generations gave no indication that they were transmitted. Whether true mutations would result if the germ cells could be reached by alpha particles is a matter of speculation. In investigation of the effects of alpha radiation upon chemical reactions in colloids, however, alpha particles, since they travel in straight lines, have proved more destructive or effective, as the case might be, than either beta or gamma radiation.

39. *The Relation of the Induced Mutation Rate to Different Physiological States in Drosophila melanogaster: IV. The Effect of Age:* FRANK BLAIR HANSON and FLORENCE HEYS, Washington University, Saint Louis. In investigation of the manner in which radiation effects are produced, the authors have undertaken a study of the influence of various internal factors acting along with radiation. The fourth of this series dealing with radio-physiology is concerned with the effects of age. The same intensity of radiation seems to produce dissimilar effects upon cells in different phases of their activity or of their life histories. Young males of Drosophila, irradiated immediately after the attainment of maturity, give a high rate of lethal mutation. The C1B method was used as a means of detecting the occurrence of lethal mutations. Males of gradually increasing age, which had been isolated throughout the aging period, were subjected to exactly the same x-ray dosage measured in time and total r-units. The consequent mutation rate decreases progressively with age: young males, 13.7795 per cent. $\pm$ 0.8418; six-day males, 11.2745 per cent. $\pm$ 0.7480; twelve-day males, 8.9286 per cent. $\pm$ 0.6879; eighteen-day males, 5.7811 per cent. $\pm$ 0.5521; twenty-four-day

males, 2.1403 per cent. ± 0.3366; thirty-day males, 0.5599 per cent. ± 0.1682. It appears from these observations that the radiosensitivity of the germ cells decreases consistently with advancing age. The experimental work is still in progress.

40. *The Operation of a Lethal Factor in Apotettix eurycephalus Hancock (Grouse Locusts):* ROBERT K. NABOURS and LAUREL L. KINGSLEY, Kansas State College, Manhattan. An autosomal lethal factor in *Apotettix eurycephalus* Hancock has been found to be located between the locus, or loci, of the group of dominant elementary color patterns B, H, M, Y and Ymf, and the locus, or loci, for the group O, R, Z and W, and somewhat nearer the former, and all on the first, smallest pair of chromosomes. The embryos carrying the homozygous lethal develop apparently normally till within 4 or 5 days of hatching and then invariably die. The individuals heterozygous for the lethal survive significantly better than their sibs homozygous for its normal allelomorph. The effect of the lethal, however its operation, is to aid viability and vigor when there is not so much (only one dose) of it, and discretely to limit life to the stage 4 or 5 days before hatching, when there is too much (a homozygous dose) of it. It may not be unreasonable to suggest that factors of this kind are sometimes responsible for hitherto unexplainable variations in viability, vigor and longevity in foetal as well as postnatal domestic animals and man.

41. *The Inheritance of Pollen Color in Petunia:* MARGARET C. FERGUSON, Wellesley College. Methods to be observed in any careful study of pollen color are outlined. *Petunia axillaris* is characterized by yellow pollen, *P. violacea* by blue pollen. Evidence is presented leading to the conclusion that 4 allelomorphic pairs of factors are operative in the inheritance of pollen color in the hybrids of these species, rather than a single pair of factors, as asserted by earlier writers for "diploid petunias." The origin of pollen color in a cultivated strain of petunia breeding true for green pollen is discussed, especially in reference to the two species named above; since these are the species that are believed to be the progenitors of practically all garden petunias.

42. *Pattern Inheritance in Cucurbita:* EDMUND W. SINNOTT and HELEN HOUGHTALING, Barnard College, Columbia University. In previous studies, fruit shape in Cucurbita has been represented by an index, the ratio of the polar to the equatorial

diameter. Types similar in index, however, may be quite different in actual shape or pattern. There are two pure lines in which the fruits are nearly isodiametric and thus have essentially the same index, but in which the pattern of the longitudinal fruit section is quite different, one being ovate and the other obovate in outline. Each type differs by a single gene (*a* and *b*, respectively) from lines with a flattened (disk) fruit shape, *AB*. In the F_2 crosses between each of these "sphere" lines and disk, the segregating "spheres" resemble their particular parental "spheres" not only in index but in specific pattern. In the F_2 of crosses between these two "spheres" there are 9/16 disks, 6/16 "spheres" and 1/16 elongates, but among the "spheres" (all of which are essentially similar in index) these two definite patterns, ovate and obovate, can readily be distinguished and are found to occur in approximately equal numbers. Evidently, each of these genes affects not only a ratio between two dimensions but an entire pattern, involving much more complex growth relationships. Just what this pattern is to be, however, depends not only upon a specific gene but upon the entire genetic complex of which this gene forms a part.

43. *Crossing-over in the Evening Primrose, between Crinkled Foliage (mut. bullata) and Double Flowers (mut. supplena):* GEO. H. SHULL, Princeton University. To be most useful in genetical analyses linked genes must be in coupling phase, that is, they must be included in the same chromosome. When closely linked genes are found in repulsion phase it may require much work to secure the crossing-over which transfers the genes in question from repulsion to the desired coupling phase. The heavily crinkled *Oenothera* mut. *bullata* originated in a single-flowered yellow strain of *Oenothera Lamarckiana*, while the gene for double flowers (mut. *supplena*) arose in an old-gold flowered, normal-leaved strain of the same species. Success in getting crossovers between old-gold and *bullata* was reported several years ago. This crossing-over occurred in a single-flowered biotype. Four years' work has given me this year for the first time crossovers between doubleness and crinkled foliage. This success has involved the growing of 230 cultures, comprising a total of over 23,000 individuals. Of these 230 cultures only two showed the result of crossing-over, suggesting a linkage between these two genes of the general order of 0.87 per cent. Unfortunately, both of these crossover progenies came from yellow-

flowered parents, so it is necessary to continue the search for an individual which shall have the five known genes of the third linkage group in a single chromosome.

44. *Inheritance of Elongate-lobed Flowers in Petunia:* E. E. DALE, Union College, and W. C. STEERE, University of Michigan. Elongate-lobed Petunia are characterized by, (a) large flowers with elongated corolla lobes and large sepals, and (b) somewhat succulent leaves and stems. Selfed elongate-lobed gave 156 elongate: 82 normal. Elongate ♀ × normal ♂ gave 64 elongate: 76 normal. Normal ♀ × elongate ♂ gave 47 elongate: 119 normal. The progeny ratios of selfed elongate and elongate ♀ × normal ♂ indicated that elongate plants were heterozygous (*Ee*) for a dominant gene *E* and that *E* was lethal when homozygous. The reciprocal crosses with normal indicated a ratio of 1*E*: 1.2*e* for the female, and 1*E*: 2.5*e* for the male germ cells of elongate plants. Disturbance of the expected 1:1 ratios might have been due, (a) to a sublethal heterozygous effect of *E* with elimination of some *Ee* zygotes, or (b) to differential growth of *E* and *e* pollen tubes with partial elimination of *E* types. Assumption (a) seems inconsistent with the data from selfed *Ee* plants and with the deficiency difference exhibited by the reciprocal crosses of *ee* × *Ee*. Differential pollen tube growth is normally associated with chromosomal unbalance. Elongate Petunias, however, have normal (2n = 14) chromosomes. It seems probable that *E* involved both a lethal effect when homozygous and a differential effect on pollen tube growth in normal (*ee*) pistils with partial elimination of the *E* germ cells.

45. *Effect of Carbohydrate Deficiency upon Meiosis in Plants with Particular Reference to the Tomato:* FREEMAN S. HOWLETT, Ohio Agricultural Experiment Station, Wooster. The carbohydrate deficiency was induced by growing the plants in an excessive nitrogen supply under the low light conditions in London, England, from October to December. The temperature of the greenhouses ranged from 65–70° F. Additional illumination was given to certain plants. Clonal series were also used during the course of the experiments, depending upon the severity of the carbohydrate deficiency, the flowers ranged in size from those which failed to reach anthesis to those which appeared to open normally. The stages of microsporogenesis as well as mature pollen grains were examined. Pollen germination tests on sugar-

agar and Knop's solution and on the stigma were carried out. Self- and cross-pollination tests were made in a considerable number of cases. In the flowers which reached anthesis pollen sterility varied in proportion to the degree of carbohydrate deficiency and reached 100 per cent. in the flowers of the most carbohydrate deficient plants. This sterility varied from one part of an anther to another, from anther to anther, flower to flower and cluster to cluster on the same plant. Examination of microsporogenesis showed that in general the meiotic divisions were quite regular but degeneration occurred immediately after or shortly after telophase II. The four nuclei thus degenerated *in situ* before cell walls were laid or following the deposition of the walls. One to all four cells degenerated, depending upon the severity of the deficiency. This work shows the importance of another environmental factor in producing male sterility and the desirability of constant and known environment conditions in certain genetic and cytological experiments.

46. *Breeding Non-broody and Intense Broody Lines of Rhode Island Reds:* F. A. HAYS, Massachusetts State College, Amherst. Non-broody and intense broody lines of Rhode Island Red pullets were carried through nine generations. An attempt was made through selection to eliminate broodiness in one line, while developing a line that would breed true for the characters; early sexual maturity, no winter pause, high intensity and high persistency. In the other line an effort was made to retain a high degree of broodiness, while developing a line similar to the first in the four high fecundity traits. Data were obtained on 259 birds in the non-broody line and 194 in the broody line. These data show that the two lines are essentially identical in age at sexual maturity, in winter intensity and in persistency. There is, however, a significant difference in the two lines in winter pause duration. In fecundity the non-broody line is superior with a mean winter egg production of 81.42 ± 1.135, compared with $69.08 \pm .974$ for the broody line. Annual egg records are also decidedly higher in the non-broody line. The means are 209.81 and 179.02, respectively, for the two lines. The data show further that degree of broodiness is inherited and that extensive progeny testing of males offers the only workable means of eliminating the broody instinct entirely.

47. *A Study of the Distribution of Species-specific Substances*

in Dove Hybrids: M. R. IRWIN, University of Wisconsin. The absorption of the agglutinins of anti-Pearlneck (*Spilopelia chinensis*) serum by erythrocytes of the Ring dove (*Streptopelia risoria*), with subsequent agglutinations, permits the identification of antigenic substances particular to the cells of the Pearlneck genus. Exhaustion of anti-Pearlneck serum by the cells of the F_1 hybrid completely removes the agglutinins for the backcrossed progeny of the hybrid mated to Ring doves, and for all succeeding backcrossed generations as well. Presumably, the antigenic cell components are determined by the chromosomal make-up of the individual bird. By a further extension of the absorption and agglutination techniques, it has been possible to distinguish differences between the cells of 38 birds of the first backcrossed generation ("one fourth Pearlnecks") with definite qualitative differences between 20 or more. Studies of the cells of progeny of successive backcrosses to Ring doves have revealed a further division of these specific Pearlneck substances. This work shows clearly that the blood cells of an individual of the Pearlneck genus are composed of a large number of distinct antigenic units, since the immune serum was produced by injections of erythrocytes from one bird. In effect, the biochemical composition of the blood cell is a quantitative character which has been divided into many of its specific parts. On the basis that these species-specific substances are produced by direct action of specific genes, their separation parallels the distribution of chromosomes. The relationships between the cells of any hybrid parent and its backcrossed offspring give pertinent evidence that the chromosomes originally derived from the Pearlneck parent tend to go together more often than expected by chance assortment.

48. *Ulna-radius Index Associated with Chondrodystrophy in Dogs:* E. M. VICARI, Cornell University Medical College, New York City. Chondrodystrophy of the leg of the Bassethound was tested on three breeds of dogs with normal leg growth of different lengths, British Bull, Saluki and the German Shepherd. One hundred individuals were obtained in the second filial generation. The ulna-radius length in the first filial generation is intermediate between the parental lengths in all three crosses, and chondrodystrophy may also be expressed as intermediate, since it is not as well marked as in its Bassethound parent. The results of the second filial generation strongly indicate a one

unit-character difference between the parental types, the dominant short chondrodystrophic Bassethound ulna-radius and the tall non-chondrodystrophic ulna-radius. The parental types are recovered in one half of the individuals. This is especially true of the Bassethound-Shepherd cross. In the Basset-Saluki cross more of the Saluki type have been recovered than of the Bassethound type. In the Bassethound-British Bull cross 23 per cent. individuals show a non-chondrodystrophic ulna-radius size, which is longer than that of the British Bull parent. This is suggestive of a recombination of the hound ulna-radius length and non-chondrodystrophy.

49. *Life Curves of Rats Deprived of Vitamin D and Their Relation to Inheritance:* JOHN W. GOWEN, Rockefeller Institute for Medical Research, Princeton, N. J. Marked inherited differences appeared in the life curves of eleven genetically differentiated lines of rats when fed a diet deficient in vitamin D. The effect of the inheritance amounted to nearly that of all other factors combined, since practically half of the squared variation in the duration of life was removed when the effect of the inheritance was made constant. The colony was fed an adequate stock diet. It was known to be free of such diseases as paratyphoid and intestinal nematode worms, etc. All animals on test were kept and fed in raised screen-bottomed cages from the 46th day of age to their death. The vitamin D deficient ration used was Steenbock's 2965. Study of the inheritance of other characters revealed a like inheritance of litter size and initial weight. By making these factors constant between different lines, it became evident that the inherited characters which largely controlled the duration of life under this diet were these characters, litter size and a growth stimulus capable of reaching a given initial weight.

50. *Duration and Extent of Irritation versus Genetic Constitution in the Etiology of Malignant Tumors:* M. R. CURTIS, W. F. DUNNING and F. D. BULLOCK, Institute of Cancer Research, Columbia University. Recently the authors have shown that hereditary factors influence the occurrence of malignancy in the walls of *Cysticercus* cysts in the rat's liver, as they determine the longevity of the host and its susceptibility to *Cysticercus* disease. It was further shown that the greater the number of cysts in a host the greater was the probability that the host would develop *Cysticercus* sarcoma and the shorter was the time interval before

the tumor appeared. It would therefore seem that each cyst had a probability to malignancy, dependent upon the age of the inclosed parasite. Further analysis showed that the proportion of tumor cysts among all cysts increased at a slowly decreasing rate with the increase in the age of the parasite and that at any age the proportion of tumors did not vary significantly with the sex of the host or with the tumor history of the immediate parents or more remote ancestors of the host. However, the probability for an individual cyst to become malignant at any age did increase with a decrease in the number of cysts per host, reaching its maximum with a single cyst.

51. *Individuality and the Hereditary Process in Mammals:* C. C. LITTLE, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. Two factors of importance complicate experiments in mammalian genetics. The first of these is the fact that individuality in mammals is a relative rather than exact term. It has a distinctly durational aspect. The variation of functions in very young and very old individuals is greater than in those at the peak of their physiological effectiveness. A typical example of this is seen in the variation in litter size among females of a closely inbred strain of mice. The existence of such variation means that ratios either in crossing-over or in the genetic study of morphological or physiological characters must be calculated with especial reference to the age of the parents. The second factor is the existence of extra-chromosomal influence demonstrated by four independent experiments conducted over a three-year period by members of the staff of the Roscoe B. Jackson Laboratory. These experiments show clearly that in the problem of the inheritance of tendencies to form spontaneous mammary tumors in mice, both chromosomal and extra-chromosomal influences are operative. The existence of these two complicating factors does not lessen the importance of the orthodox theory of chromosomal inheritance. It does, however, clearly indicate that they must be considered as essential principles to be properly evaluated before the orthodox interpretation is applied as a universal and sole explanation of results obtained in mammalian genetics.

52. *Linkage Tests of the New Shaker Mutation with Other Factors in the House Mouse (Mus musculus):* WM. H. GATES, Louisiana State University. In the fall of 1930 Madame Zavad-

skaia, of Paris, France, kindly brought over with her some mice showing a behavioristic mutation which originated in her colony. This mutation resembled in every particular the mutation of "Shaker" described by Lord and Gates (1929). On test, however, it proved to be different and probably differently located. This new shaker, sh_2, was tested for linkage against agouti (A), pink eye (p), dilution (d), short ear (se), black-eyed-white (W) and albinism (c). With the exception of the agouti and black-eyed-white cross, all tests were made by using the F_2 generation. In the case of agouti, some back-cross tests, non-agouti to agouti, were made, while in the case of the black-eyed-white cross the dominant character of black-eyed-white naturally produced a back cross. Only a few individuals were heterozygous for albinism, so only the two albino classes could be considered. In no case was the ratio significantly different from expectation of the normal Mendelian ratio. On this account the back-cross test was not made. This new shaker mutation appears to be independent of and segregates normally from the characters of agouti (A), pink eye (p), dilution (d), short ear (se), black-eyed-white (W) and albinism (c). The actual results are as follows: Agouti-shaker F_2 ratio 177 : 49 : 73 : 32; F_1 non-agouti shaker crossed to F_1 agouti shaker 139 : 57 : 153 : 54; pink-eye-shaker F_2 ratio 470 : 171 : 165 : 49; density-shaker F_2 ratio 493 : 154 : 142 : 65, short-ear-shaker F_2 ratio 499 : 153 : 151 : 68; black-eyed-white-shaker back cross 36 : 32; albino-shaker F_2 ratio 17 : 5.

53. *Two New Mutations in the Deer Mouse:* R. R. HUESTIS and ELIZABETH BARTO, University of Oregon. In connection with an experiment involving the crossing of *Peromyscus maniculatus rubidus* with *P. m. gambeli* two new characters appeared in F_3 segregants. One is a coat color similar to silver agouti Mus. The other character corresponds to flexed tail in Mus. Our data support the inference that genes for the two characters were present in trapped specimens of *P. m. gambeli*, 16 of which were used as parents. It is interesting and perhaps significant that although comparable numbers of several other geographic races have been used, in the breeding work of several investigators, *P. m. gambeli* has produced most of the mutations.

54. *Wild Types in the Platyfish:* MYRON GORDON, Cornell University. Collections in Mexico (1932) of 101 live platyfish (*Platypoecilus maculatus*), plus 68 wild specimens previously

observed, reveal that in this species there is no one wild type. In terms of phenotypes the 169 platyfish fall into forty pattern combinations. When complex combinations are tested with known recessives, they break up into about twelve basic types. The names, their genetic symbols and the frequencies with which each type occurs, are as follows: stippled (St) 169, one-spot (O) 60, twin-spot (T) 18, twin-streak (Ts) 24, moon (M) 7, moon minus satellites (Mm) 16, crescent complete (C) 24, crescent only (Cc) 15, spotted (Sp) 41, black or niger ? (N) 7, spotted dorsal (Sd) 7, spotted belly (Sb) 1. The above patterns are due to melanophores. A few live specimens showed light red body color (R) ? and some showed light red dorsal fins (Rf) ? It is believed that bright color types occur rarely in nature. In *Genetics* (1927) Gordon showed that the difference between the stippled and the goldplaty was due to an autosomal factor: stippled (StSt) dominant over gold (stst). Factors Sp, R and N are sex linked. Factors O, T, M and C are autosomal, dominant and allelomorphic (*Journal of Heredity,* 1931). Bellamy recently confirmed the autosomal nature of gold (st) but prefers to designate gold as gg and its wild allelomorph (StSt) as ++ (*Genetics,* 1933). The so-called wild type, stippled or white, StSt or ++, occurs alone only eleven times out of 169 wild caught specimens. Since the platyfish has many wild types (all dominant) it seems advantageous to indicate which wild type is involved in a cross by using a specific symbol for the particular wild type contrasted.

55. *Chromosome Numbers in Xiphophorine Fishes:* BERNARD FRIEDMAN and MYRON GORDON, Cornell University. The fishes of the genera *Platypoecilus* and *Xiphophorus* constitute the tribe Xiphophorini. The six species of xiphophorine fishes may be regarded as geographical species, as they occupy distinctive drainage areas in Mexico. The chromosome numbers in males of all the species of the tribe Xiphophorini were determined at the first maturation division in males. In *Platypoecilus couchianus, P. xiphidium* and *P. maculatus,* and in *Xiphophorus hellerii* and *X. montezumae* the haploid number of chromosomes is twenty-four. In *P. variatus* the haploid number is twenty-five, the additional univalent chromosome appearing smaller than the bivalents. The chromosomes of F_1 hybrids between *P. variatus* and *P. maculatus* are described. The extra chromosome may, together with another *variatus* chromosome, associate with a

maculatus chromosome to give a trivalent, or it may remain unpaired as a univalent. The chromosomes of these species are small and the complements they form are closely similar in appearance. Therefore no cytological characters were found that distinguish the various species of the tribe Xiphophorini, excepting *P. variatus.*

56. *The Conjugation of Double Monsters with Free Individuals of Paramecium caudatum; a Method of Identifying Crosses of Pure Lines:* CHARLES F. DEGARIS, Johns Hopkins University. In the course of experiments to produce double monsters in *Paramecium caudatum* a slide-culture containing many dividing cells was placed in a moist-chamber surrounded by ice and allowed to stand over night. When examined the next morning the preparation contained, besides free individuals, two normal conjugating pairs and five double monsters. Each of the monsters had one or two cells adhering as if in conjugation. Subsequent production of double monsters by cyanide vapor and the introduction of these monsters individually into separate cultures of conjugating organisms resulted in each monster conjugating with one or usually two free individuals. It seemed apparent that the use of double monsters might provide a definite means of identifying, or, as it were, tagging, individuals of one line in crossing with another. All monsters noted above were produced from the same lines with which they conjugated. The question arose: Would monsters of any line conjugate with free individuals of any other line, provided the latter were ready for conjugation? Repeated experiments answered this question in the negative. The monsters, in order to effect conjugation, must be derived from lines which are themselves ready for conjugation. While this circumstance greatly narrowed the application of the method, it was still of utmost service when two or more different pure lines were found to be approaching conjugation at the same time, individual monsters of one line being allowed to conjugate in mass slide cultures with free individuals of another line.

57. *Genetic Results Expressed by Fission Rates Following Conjugation between Double Monsters and Free Individuals of Paramecium caudatum:* CHARLES F. DEGARIS, Johns Hopkins University. The use of double monsters as means of identification in the crossing of pure lines of Paramecium depends on the fact that most monsters give off free progeny at each end, and

on the further fact (*Journal of Experimental Zoology,* Vol. 49, p. 133) that the genetic constitution of such progeny is not altered by the experience of monster formation. Continuing the study of race-crossing, the fission rate and cell length were still employed as adequate measurable characters. Though these characters may be somewhat changed by a period of endomixis, prolonged observation indicates that such change is not permanent. Regarding change of fission rate from conjugation, the following experiment offers striking results: In line 3 (mean fission rate 0.9) a monster was produced and conjugated with two free individuals of line 11 (mean fission rate 1.09). Result: four pure lines with mean fission rates as follows: 0.61; 0.51; 1.15; 1.15. Two lines (from posterior component and mate) have identical and fairly low rates, while two other lines (from anterior component and mate) have very low rates similar to each other but distinctly different from the posterior lines and from either of the parents. These results, while more striking than most, do, like many others, exemplify biparental inheritance and the production of heritably diverse races, following conjugation between two pure lines.

58. *Genetic Results Expressed by Cell Length Following Conjugation between Double Monsters and Free Individuals of Paramecium caudatum:* CHARLES F. DEGARIS, Johns Hopkins University. Respecting cell length, it may be noted that the difficulty of assortative mating is avoided, and long mated with short, provided the monster is produced in the long line. Otherwise such mating can not be accomplished, or at least has not been in the course of the present work. The following experiments have been carried somewhat farther than most in this series: In line 19 (mean cell length 201.3 micr.) a monster was produced and conjugated with individuals of line 22 (mean cell length 93.6 micr.) with resulting progeny having the following mean lengths: 162.8 and 164.2 from anterior conjugants; 159.3 and 158.1 from posterior conjugants. The lines from posterior conjugants were crossed 71 days later with resulting progeny having the following mean lengths: 100.2, 102.9, 198.3, 193.3. It is seen that lengths resulting from the first cross are about intermediate between the very diverse lengths of the parent lines, while in the second cross there is a segregation of lengths approaching the extremes of the grandparents. In another experiment a monster in line 15 (mean cell length 121. micr.) conjugated with two free cells of line 22 (mean cell length 93.6 micr.)

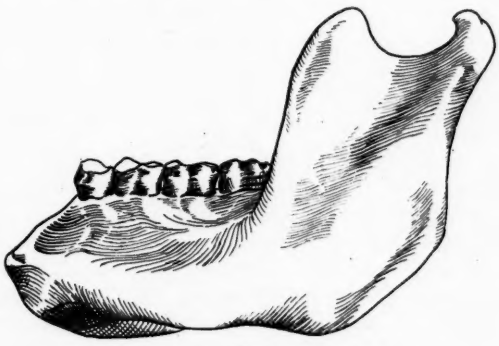
with resulting progeny having the following mean lengths: 130.5, 128.9, 97.1 100.2. The first and fourth lines were crossed 80 days later with resulting mean lengths as follows: 152.3, 147, 122.2, 119.9. From these results there appears to be a segregation of lengths at each crossing. Only 11 experiments have been carried to the second crossing, and 6 of these were inconclusive because the resulting lines were not durable. Nor is the present study of cell length in relation to conjugation as extensive as that of fission rate, because the enormous labor of fixation and microscopic measurement precluded attention of this kind to all the lines under observation. The above work on crossing of pure lines by means of monster formation continues experiments first reported in 1930 (*Anatomical Record,* Vol. 47, p. 393).

59. *The Production of Translocations and Mutations in Mice by Means of X-rays:* GEORGE D. SNELL, Washington University, St. Louis. A previous report has shown that when male mice are x-rayed with a dose of 600 r-units and mated to untreated females, approximately one fourth of their offspring are semi-sterile, producing litters averaging 1 to 4 or 5 young instead of the usual 7 or 8. The semi-sterility is due to the presence of translocations induced by the x-ray treatment. In the same experiment, recessive marker genes were used on 4 of the chromosomes of the treated P_1 males to permit the detection of induced recessive lethal mutations, or of deficiencies behaving as recessive lethals. No evidence for the induction of lethals was obtained. A total of 208 treated chromosomes from 91 F_1 individuals in the x-rayed group, and 166 untreated chromosomes from 79 F_1 individuals in the control group, were tested and found to be free from lethals closely linked with the marker genes. Whether or not the translocations have a lethal effect when homozygous remains to be determined. A large F_3 generation was raised and examined for visible mutations, the examination including an autopsy calculated to detect mutations which affected only the internal organs. One mutation was found, a variable dominant, causing a reduction in the width of the spleen, a considerable decrease in viability, and a slight stunting of growth. It is possible that this is due to, or associated with, a chromosomal abnormality, and is not a gene mutation in the strict sense. It may be concluded that whereas x-ray treatment of male mice induces the occurrence of translocations in a considerable proportion of the mature sperm, the treatment is relatively ineffective in inducing lethal or visible mutations.

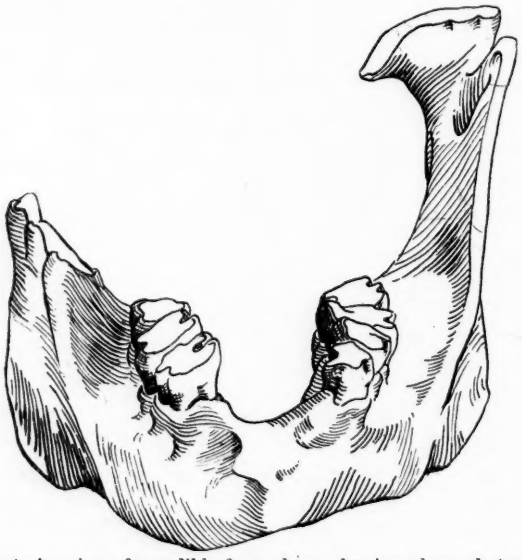
SHORTER ARTICLES AND DISCUSSION

A GORILLA SKULL WITH ABNORMAL DENTURE

THROUGH the kindness of Professor A. E. Jenks I am permitted to call attention to the unusual characters in the skull of a female gorilla at the University of Minnesota. Owing, presumably, to a gunshot wound, the anterior portion of the alveolar region of the mandible is missing, the lower denture consisting of only three molars and a premolar on the left side, and of three molars on the right side. As a result of the destruction or recession of the anterior alveolar portion of the mandible the menton region juts forward, giving rise to an apparent chin. The bony tissue in this region is rough and irregular, suggesting recovery from severe lacerations. The surfaces of the teeth show extraordinary irregularity. The inner articular surfaces of the upper premolars and first molars are much lower than the outer articular surfaces of these teeth. The articular surfaces of the second molars are nearly in the normal plane, but sharp labial cusps jut out, suggesting that they did not serve as friction surfaces. A similar prominence of outer cusps is found in all the upper teeth posterior to the canines. The articular surface of the left upper third molar slopes downward labially. An anterior portion of the right upper third molar was broken post mortem. The posterior cusps and the remaining portion of the anterior cusps of this molar are much elevated and show no signs of frictional abrasion. The roots of



View of mandible from the left.



Anterior view of mandible from above, showing planes, but not irregularities, of articular surfaces of the teeth.

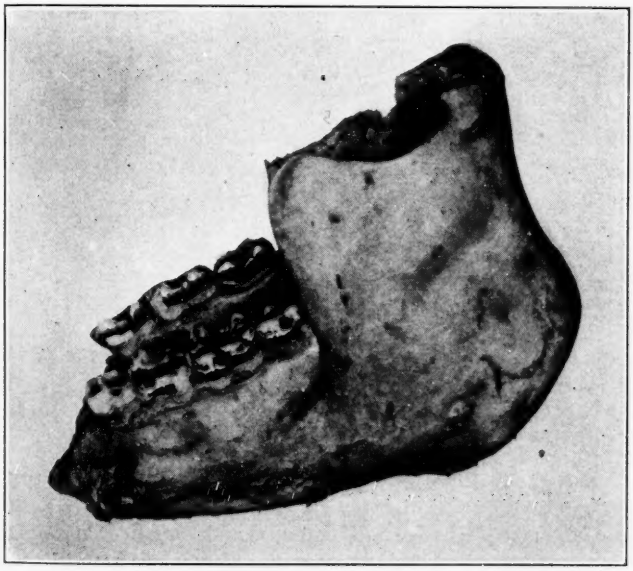
the tooth, however, are firmly embedded in their sockets. The surfaces of the teeth of the lower jaw display the converse of the characteristics of those of the upper jaw. The surfaces of the first and second molars and of the left premolar have a marked slope. The irregular surface of the left lower third molar slopes downward lingually; the surface of the right lower third molar approximates the normal surface contour. All the lower teeth, like the upper, show deep pitting and have one or more high cusps.

The upper portion of the right ascending ramus, from the region slightly above the alveolar process, is missing, but when the left condyle is fitted into the glenoid fossa it is clear that the outer articular surfaces of the lower molars and the one premolar articulated with the inner surfaces of the upper molars.

In the process of healing, the anterior portion of the mandible was considerably narrowed; as a result of this constriction the lower dental line falls well within the upper. It appears also that the lower third molar did not touch the upper teeth, except possibly on a small anterior surface of that portion of the molar

which is missing. It is clear, therefore, that for some time before its death this gorilla did not have the side to side swing of the jaw which is a characteristic of the apes. The pitting of the teeth, the upstanding cusps and the erratic slopes of the surfaces of the teeth show that the jaw worked with a scissors-like motion.

The glenoid fossae show bilateral asymmetry. The depth of the left is 9 mm, that of the right is 8 mm. The depth of the concavity of the anterior articular eminence is 9 mm on the left side, and 6 mm on the right side. In a normal female gorilla skull at the University of Minnesota the depth of each glenoid fossa is 7 mm, the depth of the concavity of each anterior articular eminence is 8 mm. I have previously pointed out that the swing of the jaw influences the depth of the fossae, and that in man the loss of anterior teeth may lead to a deepening of the fossae.[1] I have found one description of a comparable change



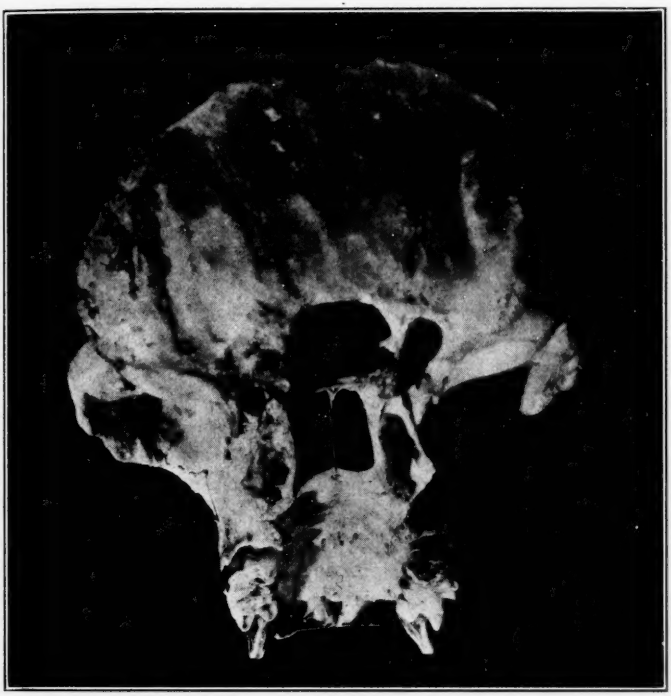
View of the mandible from the side.

[1] Wilson D. Wallis, "An Unusual Form of Glenoid Fossa possibly due to Loss of Teeth," *Dental Cosmos*, 1924; E. W. Hawkes and Wilson D. Wallis, "A Note on the Glenoid Fossa," *American Anthropologist*, 1916.

Upper denture.

in the anthropoid ape. Howell describes the skull of a gorilla in which the anterior articular eminence of the right glenoid fossa is flattened and much filled. This condition is correlated with the flattening of the right articular surface of the condyle, which is "broadened in an anterior-posterior direction, and much pitted and roughened, and the neck, as measured from the base of the coronoid process, shortened by about 7 mm. It is very likely that the functions of the abnormal side of the jaw were even more severely interfered with than can now be told from an examination of the skull, through destruction of part of the condyloid ligaments."[2] Though this instance is not completely analogous to the one described above, both suggest the influence of function upon form. In each case a presumed modification in function has resulted in an obvious modification of form.

[2] A. Brazier Howell, "Asymmetry in the Skulls of Mammals," *Proc. U. S. Nat. Museum*, 67, No. 2599, Art. 27, pp. 1–17, 1926.



Basal view of skull and upper denture.

SUMMARY

The skull described above has an apparent chin, due to marked recession of the anterior alveolar region of the mandible, as a result, presumably, of destruction of the anterior alveolar tissue. The molars and premolars, both upper and lower, are deeply pitted, and the cusps are unusually prominent. The articular surfaces are very irregular and show marked departures from the normal plane. The jaw had little side to side swing.

The comparatively deep and asymmetrical glenoid fossae, as well as the irregularities of the articular surfaces of the teeth are doubtless functionally related to the up and down movement of the jaw, which was due in part to the fact that the constricted anterior portion of the mandible forced the median line of the lower teeth well within the median line of the upper.

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THE SOMATIC CHROMOSOMES OF *CYPRIPEDIUM HIRSUTUM* AND SIX SPECIES OF THE GENUS *HABENARIA*

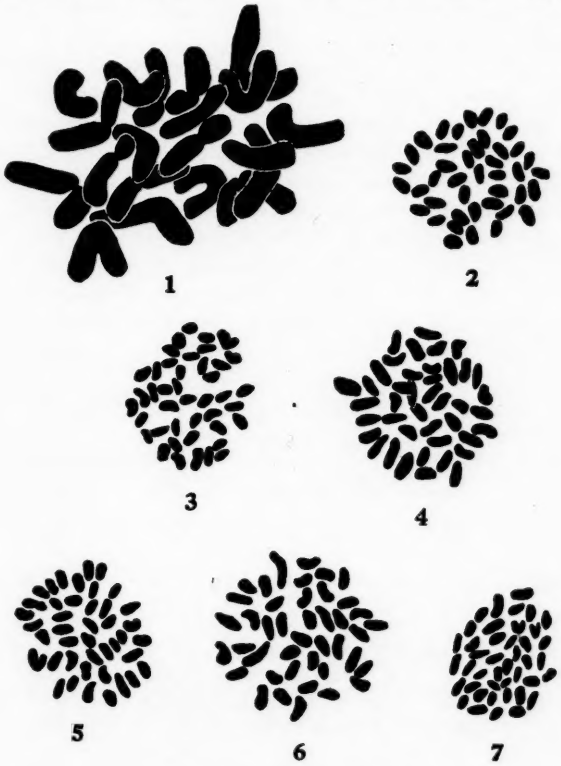
IN a recent paper reporting the chromosome numbers of some of our native orchids (Humphrey, 1932), three species of *Cypripedium* and two of *Habenaria* were studied. Two species of *Orchis* and *Calypso bulbosa* were also reported. The lady slippers, *Cypripedium acaule, C. candidum* and *C. pubescens,* each had 20 chromosomes in the somatic tissue. These chromosomes were remarkable for their great size, and in this respect they were distinctly different from all the other species studied. The two species of *Habenaria* were of special interest because the chromosomes of *H. bracteata* were several times the size of those of *H. orbiculata,* and until further studies were made it could not be known which was the characteristic condition for the genus. Thus a further study of *Habenaria* was particularly desirable. Both *Habenarias* had 42 chromosomes. *Orchis spectabilis* and *Orchis rotundifolia* likewise had 42, while *Calypso bulbosa* had 32. The chromosomes of *H. orbiculata,* and both species of *Orchis* and *Calypso* were very small.

In 1907, Pace reported the chromosome number for *Cypripedium hirsutum,* under the name *C. spectabile,* as being $n = 11$. She reported the same number for *C. pubescens* and *C. parviflorum.* The work of Belling (1924) and Humphrey (1932) showed *C. pubescens* to have $n = 10$. The present investigation leaves no doubt but that the number for *C. hirsutum* is also $n = 10$. It is anticipated that the same number will be found in *C. parviflorum* when it is reexamined. The only work on *Habenaria* was the report by Brown in 1909, in which he gave the number $n = 16$ for *Habenaria cilearis.*

The material for the present study was collected in Minnesota and Massachusetts by Mr. Murray Buell, of the University of Minnesota. *Habenaria blephariglottis* and *H. clavellata* were taken from Hawly Bog in Franklin County, Mass. The other five species came from Itaska State Park, Minnesota. All the material was killed and stored in (alcohol-formol-acetic) killing fluid until imbedded in paraffin. Iron-alum hematoxylin and iodine-violet were used for staining. A camera lucida was used to make the drawings and verify the counts.

The species included in this study were the following: *Cypripedium hirsutum* Mill., *Habenaria blephariglottis* (Wild.) Torr.,

H. clavellata (Michx.) Spreng., *H. dilitata* (Pursh) Gray, *H. Hookeri* Torr., *H. Hyperborea* (L.) R. Br., and *H. obtusata* (Pursh) Richards. An examination of the plate will reveal the size and numerical relationships of the chromosomes. *Cypripedium hirsutum* (Fig. 1) has twenty chromosomes. As in the



EXPLANATION OF THE PLATE

A Zeiss Microscope with a 90 × 1.4 N.A. objective and 20× oculars was used, and the drawings were made with the help of a Bausch and Lomb Camera Lucida. All figures are magnified 1,700 times. FIG. 1. The somatic chromosomes of *Cypripedium hirsutum* 20; FIG. 2. The somatic chromosomes of *Habenaria blephariglottis* 42; FIG. 3. The somatic chromosomes of *H. clavellata* 42; FIG. 4. The somatic chromosomes of *H. dilatata* 42; FIG. 5. The somatic chromosomes of *H. Hookeri* 42; FIG. 6. The somatic chromosomes of *H. hyperborea* 42; FIG. 7. The somatic chromosomes of *H. obtusata* 42.

other species of this genus the chromosomes are very large. Each species of *Habenaria* (Figs. 2–7) had 42 chromosomes, and they are all quite similar in size and shape. The chromosomes of these six species are morphologically very similar to those of *H. orbiculata* previously reported. Evidently the small size is characteristic for the genus, and the larger size of the chromosomes of *H. bracteata* is exceptional.

It is interesting from an evolutionary standpoint that *Orchis* and *Habenaria* not only have the same chromosome number, but that their chromosomes are morphologically similar. Both of these genera are in the same tribe (*Ophrydeae*) and are much alike in many respects. The large size of the chromosomes of *H. bracteata* may be a genetic variation of chromosome size. *Cypripedium* is a tribe in itself and is distinguished by the lower number, but great size, of its chromosomes. Thus cytologically as well as phylogenetically it is far removed from *Orchis* and *Habenaria*, which are very similar in their relationships.

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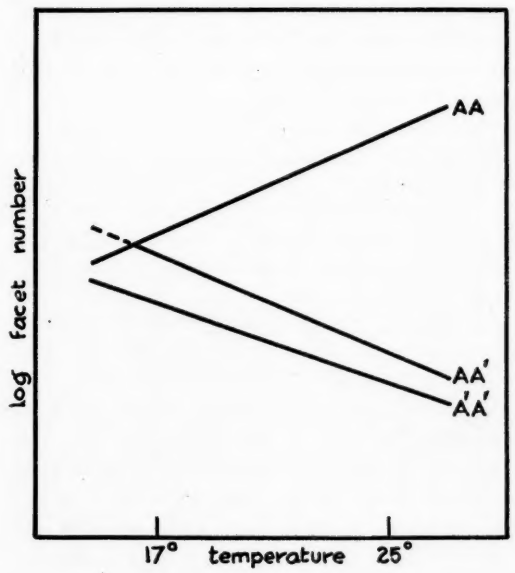
ON MENDELIAN DOMINANCE AND THE SERIAL ORDER OF PHENOTYPIC EFFECTS IN THE BAR SERIES OF *DROSOPHILA MELANOGASTER*

THE immediate extension of Mendelism after the rediscovery of Mendel's laws in 1900 took for its main interest the analysis of the architecture of the germ plasm with results summed up in the familiar chromosome maps. For such analysis it is not necessary to have a range of an external variable, since alteration of external conditions under which development occurs does not alter the linear order of the gene arrangement. Since the problem of the genetic composition of the chromosomes is solved in principle there has been a return of interest in the problem

of dominance, both from an evolutionary point of view in regard to an explanation of the dominance of the wild type under ordinary conditions, and from an ontogenetic point of view in regard to an explanation in physiological terms of the differences in developmental processes which are causally related to the phenotypic differences exhibited by individuals with the genetic constitutions AA, Aa and aa. It is clear that the problem of dominance is related to the more general problem of the manner of action of the genes, which is further related to the problem of the nature of the gene.

Starting from the arrangement in a quantitative series of the phenotypic effects produced under one set of external conditions by a series of allelomorphs in homozygous and heterozygous state, there have been attempts to draw conclusions in regard to the nature of dominance, in regard to the manner of action of genes, and in regard to the nature of the allelomorphs, especially as to whether they differ qualitatively or whether they represent merely different quantities of the same gene substance (for discussion and references, see Stern, 1930). The main purpose of the present note is to point out the necessarily defective character of such a procedure, since the serial order of the phenotypic effects, unlike the linear order of the genes, varies with the external conditions under which development occurs.



The accompanying figure gives an excellent example of the point involved. It shows in a semi-logarithmic plot the relation of facet number to temperature in two sets of homozygous females and the corresponding heterozygous females of *Drosophila melanogaster*. It is based on the data obtained by Luce (1931) on the effect of temperature on facet number in infrabar (AA), bar (A′A′), and their heterozygotes (AA′) and on those obtained by E. C. Driver (1931) and by O. W. Driver (1931) on the effect of temperature on facet number in double infrabar (AA), ultrabar (A′A′), and their heterozygotes (AA′). It is somewhat generalized in order to serve for both sets of data. It can be seen that under standard conditions, *i.e.*, about 25°, the heterozygote AA′ is more like the parental type A′A′; dominance of A′ over A is high but incomplete. As the temperature decreases the heterozygote approaches closer and closer to the AA parental type, but it is still intermediate. Dominance of A′ over A becomes less and less until at a temperature slightly lower than 17°, the heterozygote has the same facet number as the AA parental type; A′ is completely recessive to A. Below this point, indicated in the figure by the dashed portion of the line representing the heterozygotes, the dominance of A over A′ is greater than 100; there is a heterosis-like effect. (For similar heterosis-like effects, see Hersh and Ward, 1932; Crozier and Pincus, 1932.)

If we calculate, from the formula given by Zeleny (1920), the dominance coefficients directly from the facet number at those temperatures for which the data allow it, we find that the dominance coefficient of bar over infrabar is 37.56 at 17°, 78.21 at 22°, and 94.31 at 27°. Similar dominance coefficients of ultrabar over double infrabar, using the heterozygotes from double infrabar ♀♀ × ultrabar ♂♂ matings, are found to be 0.01 at 17° and 77.13 at 27°. Using the reciprocal heterozygotes the dominance coefficient of ultrabar over double infrabar is found to be 31.50 at 17° and 79.22 at 27°.

In the short temperature interval of about ten degrees Centigrade bar passes from complete recessiveness to almost complete dominance in relation to infrabar. Wright (1929) called attention to the fact that in all possible combinations of members of the bar series at 25° (data from Morgan, Sturtevant and Bridges, 1925) infrabar is almost or quite completely recessive to bar. In the light of the data of O. W. Driver and of Luce, this relation denotes that in all combinations where there is at least one bar

factor, facet number will decrease with increase in temperature. Bar dominates the character of the relative rate of change in the facet-temperature relation. The facts known regarding the temperature-effective periods of facet determination do not allow, however, the conclusion that the recessive gene is inactivated. (At least, not in any ordinary meaning of the term inactivation.)

In regard to the serial arrangement of these genetic differences on the basis of the quantitative order of the phenotypic effects it can readily be seen from the figure that at (or quite close to) 17° the order is $AA = AA' > A'A'$; above this temperature the order is $AA > AA' > A'A'$, below this same point the order is $AA' > AA > A'A'$. If we should extrapolate to slightly lower temperatures the expected order would be $AA' > A'A' > AA$. At the higher supranormal temperatures, 30° and above, a different order would be expected.

It seems clear that any theory of dominance, or of genic action, or of the nature of the gene, based on the quantitative serial order of the phenotypic effects at 25° would be inconsistent with a theory based on the serial order of the data at 17° or below.

It is of further interest and may be of importance for such theories that such irregularities may be obviated by serial arrangement in terms of the first derivative of the function relating the phenotypic quantity to an external variable. It can readily be seen from the figure that, on the basis of the first derivative, in the present instance the order is the same at all temperatures. In terms of the algebraic increase in the first derivative this order becomes AA', A'A', AA. For the combinations of the bar series mentioned above and including such other combinations that are safe to include on the basis of present knowledge, the order, again in terms of algebraic increase in the first derivative, becomes +/+, B/+, Bi/B, B/B, BiBi/BB, BB/BB, BiBi/BiBi, Bi/Bi.

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SEX IN DROSOPHILA AND OTHER ORGANISMS

IN a recent note Professor Punnett[1] has raised the question whether a fundamental difference exists between the mode of sex-determination in Drosophila and in other organisms. He is concerned with the distinction between the point of view which assumes a "primary" sex factor with a constellation of modifiers and that which assumes the sex of an individual to be due to the interaction of many genes, no one of which can be regarded as "the" sex factor.

We have endeavored to prove[2] that sex determination in *Drosophila melanogaster* conforms to the latter scheme, by showing that no section of the X-chromosome of this species is in itself sufficient to transform a male into a female, and that the sexual effectiveness of a fragment of the X is a function of its length. Even from Punnett's own analysis of our data it follows that at least three sex-factors must be present in the X-chromosome—one at the yellow end, one in the middle and one at the right end. Further discussion of this point is unnecessary here, since we have in the press (*Journal of Genetics*) an account of the extension of our previous work.

[1] AMER. NAT., lxvii, pp. 473–476, 1933.
[2] *Proc. Nat. Acad. Sci.*, xvii, pp. 513–518, 1931.

A comparison of Drosophila with other organisms is made difficult by the fact that critical data bearing on the problem are available for very few forms. The two cases which Punnett cites as contrary to the Drosophila formulation—Lebistes and Lymantria—are not really so at all. This is especially evident in the light of Winge's recent findings in Lebistes. Winge[3] has obtained a strain of this fish in which, as a result of mutation, the rôle of "sex determiner" has been taken over by a former autosome. It is evident, therefore, that at least two genetic factors are concerned here with sex-determination. Goldschmidt's data on Lymantria and Witschi's on Rana are also best interpreted by assuming a multiplicity of the sex-determining factors. There are, in fact, no established cases of sex determination by a single genetic factor.

In conclusion, a word on definition. Punnett chooses to define modifiers as having "no influence on the phenotypic character associated with the factor modified unless this factor be present." Perhaps a more objective statement of the facts is that the influence of the modifier is detected only under certain stated conditions. On such a basis, the distinction between "main factor" and "modifier" is sometimes useful for practical purposes, but it seems to lack significance in general theory.

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A NEW METHOD OF ETHERIZING DROSOPHILA IN MASS

ONE of the most time-consuming things in making counts that involve mere inspection of large numbers of families separately grown in vials is the etherization of each family. I have devised a method by which the flies in several hundred vials can be etherized in one operation. At the same time the method makes possible the convenient and leisurely examination of all the flies, without over-etherizing or killing any of them.

The method in brief consists in etherizing the flies through the cotton stoppers. The vials are placed upside down in a box (containing about 200 of the vials). Bits of cotton, each with

[3] *Proc. VI, Internat. Congress Genetics*, 1, pp. 343–355, 1932.

several drops of ether, are distributed at about ten evenly spaced places in the box (between the vials), and not too deeply inserted. The cover (which is snug-fitting) is then placed on the box. After the lapse of about ten minutes, the cover is removed. The flies will now be found resting on the cotton stoppers of the vials (the latter having previously been turned upside down). The flies themselves can be easily removed and examined by simply removing the cotton stopper from the vial.

The flies thus etherized will remain under the ether, without injury to themselves, for as long as two hours (inside the vials, a quarter to a half hour outside). One can therefore examine one or two hundred of the vials at his convenience. The flies thus etherized can be used for further matings. Their wings do not fold up, as they do in over-etherized flies.

Why flies, etherized by the above method, should remain so long under ether, without injury to themselves, is a question which I can not answer with certainty at present. It seems that the flies get just the right amount of ether through the cotton stoppers for their complete anesthetization; not an excessive amount, as in the ordinary method.

Incidentally, the boxes in which the vials are placed are very convenient, not only for the etherization of the flies, but also for making up the vials with food, for making the matings and for keeping the vials together while the flies are breeding. I have been using boxes divided into five or six compartments (by tin partitions). Each compartment is just broad enough to contain two rows of vials and long enough for fifteen to twenty vials. It is convenient to have a cover permanently attached by means of hinges. The boxes are first filled with the empty vials, and food is added to the vials. The covers of the boxes are then closed (and held down firmly by means of a little holder permanently attached to the box). After the food has cooled, the vials are used for matings without removing them from their boxes. While the flies are breeding, the covers of the boxes are kept slightly open (by inserting an empty vial on its side between the top of the box and the vials). The flies are etherized in the same boxes, but it is necessary to turn the vials upside down before etherizing the flies, as already mentioned.

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